A NEW SPECIES OF *SYNGAMUS* (*S. AURIS*) FROM THE MIDDLE EAR OF THE CAT IN FOOCHOW, CHINA[1]

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(With 9 Figures in the Text)

INTRODUCTION

DURING a survey of the helminth fauna of the domestic cat in the immediate vicinity of Foochow, Fukien Province, China, the junior author discovered paired roundworms in the vestibule of the middle ear of this host. These parasites were first found in 1932 and again in the spring and fall of 1933. There appears to be no seasonal infection. Of forty-eight animals sacrificed eleven (22·9 per cent.) were found to be infected.

These parasites live in the *cavum tympani*, occupying either or both chambers. They were once observed passing from one chamber to the other through the small cleft in the intervening septum. As many as six pairs have been recovered from one ear. They have never been found attached to the wall of the chambers, although the haemorrhagic condition of the membrane of the wall, as well as the amount of freshly ingested blood in the worms, particularly the females, indicates that their food supply is undoubtedly obtained from this host tissue. On the other hand, the tympanic membrane, both on its inner and outer aspects, was always intact and showed no injury. During life the female is a brilliant blood red; the male, a lighter orange red. They are very active and have a very distinct light-avoiding reaction. The males are much more delicate than the females and die soon after removal from the host. On one occasion a dead pair was found *in situ* in the host.

Altogether more than two dozen pairs of the worms have been observed. Of this number fourteen pairs have been fixed in a mixture of steaming 2 per cent. formaldehyde and 10 per cent. glycerine and have been made available for subsequent study. In some cases the delicate males were badly injured during manipulation before or after fixation. Some females were so engorged with blood that their internal structures were obscured. Nevertheless, an adequate number was sufficiently well preserved for careful study, which has demonstrated that the species is new to science.

DESCRIPTION OF *SYNGAMUS AURIS* N.SP.

The worms are members of the genus *Syngamus*. They are permanently joined *in copula* (Fig. 1), so that the male cannot be separated without injury

[1] Contribution from the Parasitology Laboratory, School of Medicine, Tulane University of Louisiana, New Orleans, La., and from the Foochow Polytechnic Middle School, Foochow, China.

to the bursa; the buccal chamber is provided with eight teeth (Fig. 2), and the bursal rays are short and stubby.

Six intact males were available for measurement. They ranged in length from 3·3 to 8·1 mm., with an average of 5·6 mm. The type male specimen had a length of 6·1 mm. and a greatest transverse diameter of 0·39 mm. The smaller males were not always mated with small females. The buccal capsule of the type male (Fig. 3) had a diameter of 270μ, with an orifice of 200μ and a muscular wall 224μ in diameter by 200μ deep. The bursa of the male is intimately and permanently attached (fused) with the tissues surrounding the vulva of the female (Fig. 4). It was found to be impossible to separate the worms without seriously damaging the bursa. The blunt rays of the bursa are typical for the genus but specifically differentiable (Fig. 5). The ventral rays are thick and stubby, subequal and close together. Some distance from them are the three laterals, of which the externo-lateral is considerably longer than the other two of the triad. The relatively long isolated externo-dorsal ray lies midway between the lateral group and the dorsal. This latter is practically vestigial and consists of a single bulb-like thickening with a pair of minute bifurcations. The delicate spicules (Fig. 6) are subequal, being about 52μ in length by 9μ in breadth. They were visible only after dissection of the bursal complex.

Measurement of ten intact females showed a length variation from 14 to 30 mm., with an average of 21 mm. The greatest diameter ranges from 0·8 to 1·4 mm., with an average of 1·12 mm. In the pair designated as type, in which the female had a measurement of 14 by 0·88 mm., the head (Fig. 7) had a diameter of 664μ, the orifice 320μ, and the muscular wall of the capsule 580μ in diameter by 362μ in depth. The body is essentially cylindrical for its entire length, except for the posterior tip, which is sharply pointed (Fig. 8). There is a suggestion of a pair of papillae in the caudal extremity. The vulvar opening lies a short distance in front of the middle of the body (43–46 per cent. distant from the anterior end). The uterine coils extend posteriad to the subcaudal region of the body.

The recently laid eggs of this species (unpreserved material) measure 88

Legends to Figs. 1–9

Fig. 1. Paired female and male *Syngamus auris*. × 9. *Note* position of vulva.

Fig. 2. Head-on view of female *S. auris*. × 58. *Note* symmetry of the eight dental processes and the broad dorsal and ventral sectors of the capsular rim.

Fig. 3. Anterior end of male worm, lateral view. × 58.

Fig. 4. Lateral view of male's attachment to female, showing bursal rays visible from lateral aspect. × 58.

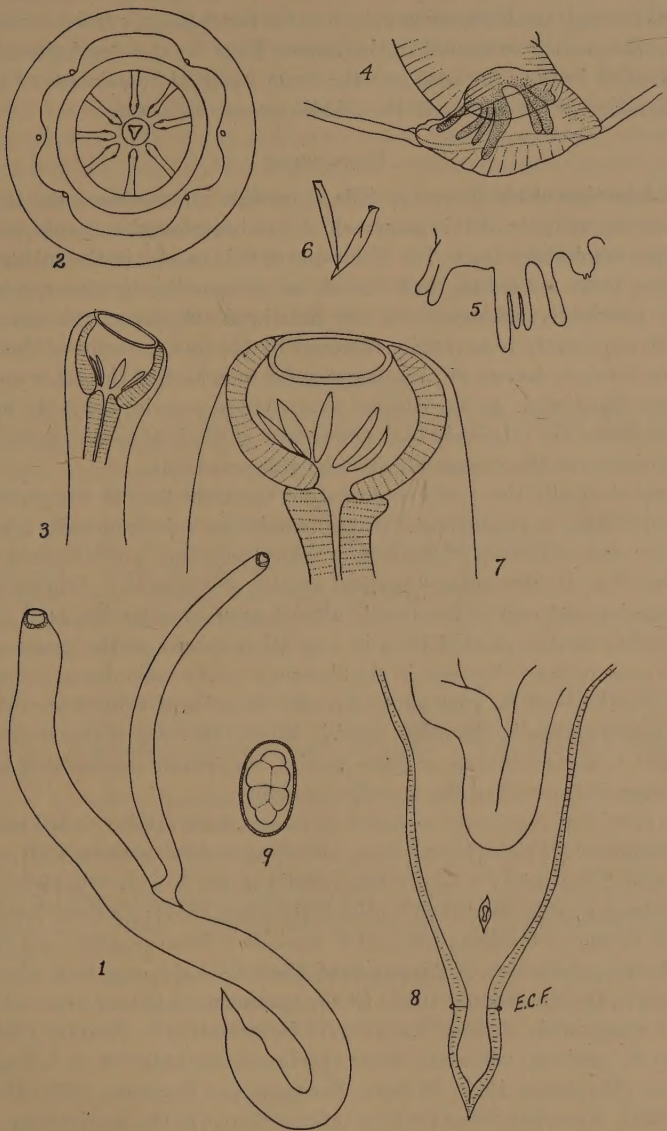
Fig. 5. Flattened projection of bursal rays of male worm (one side only).

Fig. 6. Copulatory spicules of male worm. × 210.

Fig. 7. Anterior end of female worm, lateral view. × 58.

Fig. 8. Posterior end of female worm, ventral view, showing caudal papillae, anal opening and posterior coil of uterus. × 58.

Fig. 9. Egg of *S. auris* from vulvar vestibule. × 165. *Note* characteristic sculpturing but absence of opercula.



Figs. 1–9

by 48μ, and have a thick transparent shell, with characteristic sculpturing, but *without opercula* (Fig. 9). They are in the 4- to 8-cell stage when laid. They pass out through the Eustachian tube into the nasopharynx and are swallowed, being subsequently recovered in the faeces. Thus far the development of the embryo after leaving the host and the exact mode of inoculation of the host and migration of the worms to the middle ear are unknown.

Discussion

Examination of the literature fails to provide information concerning any other known member of this genus which has been found to reside outside of the upper respiratory tract. The discovery of this species in the middle ear is, therefore, most interesting and reveals an adaptation by the species to a habitat previously unclaimed by any helminth. However, this site of predilection apparently is as readily achieved by the larval worms of this species as is the trachea, larynx or nasopharynx for members of the other species of the genus *Syngamus*. In the isolated geographical area in which the infection has been found the relatively high percentage of the host species parasitised is itself an index of the success attained by this nematode.

Morphologically the worm conforms for the most part to the generic concept; specifically it is different. In the female the vulvar opening is situated 43–46 per cent. of the body length from the anterior end, which is much farther posterior than in any other described species of *Syngamus*. Compensatorily the posteriormost coil of the uterus almost approximates the anal opening, considerably farther caudad than in any other species of the genus. In the male the ray pattern is unique in the character of the main dorsal element. In both sexes the head-on view shows broader dorsal and ventral sectors of the buccal capsule than in the other species. While other characters serve to differentiate *S. auris* from one or more species, the criteria enumerated separate this syngamid from all of the described species.

The species of *Syngamus* recorded from mammals appear to fall into three host categories: (1) those from felines, including *S. felis* Cameron 1931, *S. auris* Faust and Tang n.sp., a newly described species, *S. ierei* Buckley (1934 *a*), and the inadequately described *S. dispar* (Diesing, 1851); (2) those from herbivores, *S. laryngeus* Railliet 1899, and *S. nasicola* v. Linstow 1899, and (3) those from the hippopotamus, *S. hippopotami* Gedoelst 1924, and from the Indian elephant, *S. indicus* Mönnig 1932. Of the specimens obtained from six human cases of syngamosis, *S. kingi* Leiper 1913 is, according to Buckley (1934), the same as *S. nasicola*, while the other specimens are believed to belong to *S. laryngeus* (Travassos, 1922; St John, Simmons and Gardiner, 1929; Hoffman, 1931, 1932). None have thus far been referred to one of the species from felines, and none from the species described from avian hosts. In fact, the mammalian species are apparently quite distinct from the avian forms.

The eggs of the avian species of *Syngamus* all appear to have one or two opercula; those of the mammalian species are apparently non-operculate. If

this differentiating character is constant, it might be desirable and advisable to erect separate subgenera for these two groups of species on this basis. Certain it seems that some stable character or characters, other than their host predilections, need to be found for separating these groups.

In conclusion it is necessary to emend the generic concept for the genus *Syngamus* von Siebold 1836, as well as that of the family Syngamidae Leiper 1913, in order to include the species *S. auris*. This emendation requires incorporation of the new habitat, namely, the middle ear; likewise the presence of one or two opercula in the eggs of the avian species and the apparent absence of such opercula in the eggs of the mammalian species. Furthermore, the generic concept must be modified to include *S. nasicola*, in which copulatory spicules appear to be absent. Such limitations of definition are always to be expected as more adequate knowledge of a group becomes available.

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EVIDENCE OF THE SUCCESSFUL DESTRUCTION OF SCHISTOSOMES

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In human schistosome infections there is always the possibility that a patient may harbour a preponderance of male parasites, or that female parasites may be absent or remain undeveloped for long periods of time. This possibility is not generally recognised, in spite of the fact that the above-mentioned conditions are frequent in experimental tests. And it is evident that, if such conditions do hold for man, the mere absence of eggs from the urine or faeces is of little avail in determining the presence of an infection or in estimating the effects of treatment. Since, further, the ova are not readily detected in faeces and slight infections of the urine are demonstrable only when the very last drops of urine are collected and centrifugalised, a less uncertain and laborious method of estimation is clearly desirable. The complement-fixation test is not entirely trustworthy, though a negative result and a normal blood count would prove useful evidence of successful treatment. It is suggested in this paper that the degree of eosinophilia may afford a reliable confirmatory test.

Infections with *Schistosoma japonicum* give rise to a strong eosinophilia, and where the general health improves under treatment the gradual return of the blood count to normal has been regarded as an indication of the successful destruction of the adult schistosomes (Graham-Yool, 1934).

In *Schistosoma haematobium* infection, repeated absence of ova from the urine has usually been accepted as evidence of cure; but a persistent eosinophilia has sometimes been attributed to the presence of *Ascaria* or *Ankylostoma* in addition to the flukes. The possible survival of male schistosomes has been overlooked.

In South Africa other parasitic infections are less common in patients suffering from schistosomiasis than in Egypt, especially where *Schistosoma mansoni* is concerned; and here a strong eosinophilia develops during the stage of invasion. A persistent rise, in spite of treatment, must then indicate survival of the parasites, even where there is no sign of ova, and a subsequent sudden rise may denote a fresh infection, unless due to trichinosis.

Where infection is suspected as a result of the eosinophilia, one is justified in testing the effect of a course of intravenous injections of antimonium potassium tartrate; and where this immediately results in general improvement in the patient's condition, a gradual fall in the eosinophils, disappearance of abdominal tenderness and an increase in weight, we are justified in assuming that the worms are being destroyed. Incidentally, it is as well to make as certain as possible of the purity of the drug. Careful investigation has shown that few

drug houses are capable of producing a perfectly pure supply, a common impurity being lead (Khalil, 1926). Nevertheless, tartar emetic would seem to be more efficient than Fouadin, but so far it has not been possible to obtain a series of blood tests in patients treated with this preparation, such as are available with tartar emetic or emetine (Cawston, 1922). There is no doubt that many patients so treated have been declared free, solely on the evidence of the absence of ova from the urine or of cystoscopic examination; but such patients have not been relieved of their symptoms and may even have served to introduce the infection into fresh localities.

A phenomenon which appears to be connected with the blood changes in these infections is the development of muscular pains, especially pronounced in one or both shoulders during a course of treatment with antimony. These pains come on a few hours after an injection, and often persist till the following day; but they may last for a week or more in susceptible persons, in spite of treatment with cascara, potassium citrate, glucose and hot applications. Hot baths may even aggravate the condition, and this suggests hyperaemia of the liver as a cause. The pains may occur at an altitude of over 6000 ft. or at sea-level. Sea bathing a few hours after an injection—a not uncommon occurrence in Natal—seems to predispose to them, especially in damp weather.

There is no doubt that the pains are due to changes taking place in the liver substance, either as a result of the death and disintegration of the schistosomes or as a result of the antimony itself. This point could be settled by submitting normal patients to a course of intravenous injections of tartar emetic. Some physicians in Natal have come to expect these pains during a course of treatment and, if the urine remains free from albumen towards the end of treatment, they explain to their patients that they must put up with the pains, lest the degenerating parasites recover from the drug.

It seems to be the general experience that these symptoms occur most frequently when too large doses are being administered—an irrational method of treatment, as there is no sound reason for using the same total dose for all patients. The required dose probably varies in accordance with the response of the patient to the injections (Christopherson and Newlove, 1919). If it were the antimony which alone was responsible for the death of the schistosomes, the greater the antimony content of a drug the more effective it should be, and 30 grains of tartar emetic, given over a period of a fortnight or three weeks in persons who could tolerate large repeated doses, should cure as surely as the same dose spread over five weeks. Since too large repeated doses have the effect rather of precipitating a return of symptoms, it is concluded that successful treatment depends rather on blood changes taking place while a patient is kept under the influence of antimony.

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A NEW BLOOD TREMATODE, *PARADEONTACYLIX SANGUINICOLOIDES* N.G., N.SP., FROM *SERIOLA LALANDI* WITH A KEY TO THE SPECIES OF THE FAMILY APOROCOTYLIDAE

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(With 1 Figure in the Text)

A single specimen of a blood fluke was obtained from the gills of an amberjack or coronado, *Seriola lalandi* Cuv. and Val., taken by the writer on November 30th, 1929, from the Atlantic Ocean off Miami, Florida. This fluke belongs to the family Aporocotylidae Odhner 1912, and is regarded as the type of a new genus.

Paradeontacylix n.gen.

Generic diagnosis. Aporocotylidae. Small, slender trematodes; cuticle on ventral surface of lateral margins armed with transverse rows of spines; lateral margins of body thin and recurved ventrally for a part of their length, forming parallel folds about one-sixth as wide as the body. Suckers absent; pharynx absent; oesophagus long; intestine H-shaped. Testes numerous, arranged in two median, irregular longitudinal rows, extending from fork of crura to ovary. Ovary median, in posterior third of body. Uterus long, with few coils, postovarial. Genital pores separate, dorsal, postovarial; male pore posterior and lateral to female pore. Vitellaria extensive; unpaired median vitelline duct extending from zone of ovary to ootype. In circulatory system of marine fishes.

Type species. Paradeontacylix sanguinicoloides n.sp.

Paradeontacylix sanguinicoloides n.sp.

Description. Paradeontacylix. Body slender, 3·25 mm. long by 330μ wide; cuticle on ventral surface along margin of body provided with numerous rows of spines; twelve of the spines at posterior end of body comparatively large and conspicuous, rose-thorn-shaped, arranged in rows of three spines each, the largest about 15μ long by 9μ wide at base; spines along margin of body arranged in approximately 500 transverse rows, the number of spines per row increasing from three per row at posterior end to fourteen per row at equator of body. Suckers absent; mouth opening subterminal. Oesophagus 671μ long by approximately 30μ wide. Anterior portion of oesophagus drawn out into a narrow tubular structure having its posterior end slightly dilated, but apparently not forming a true pharynx. Intestine with four short indistinct

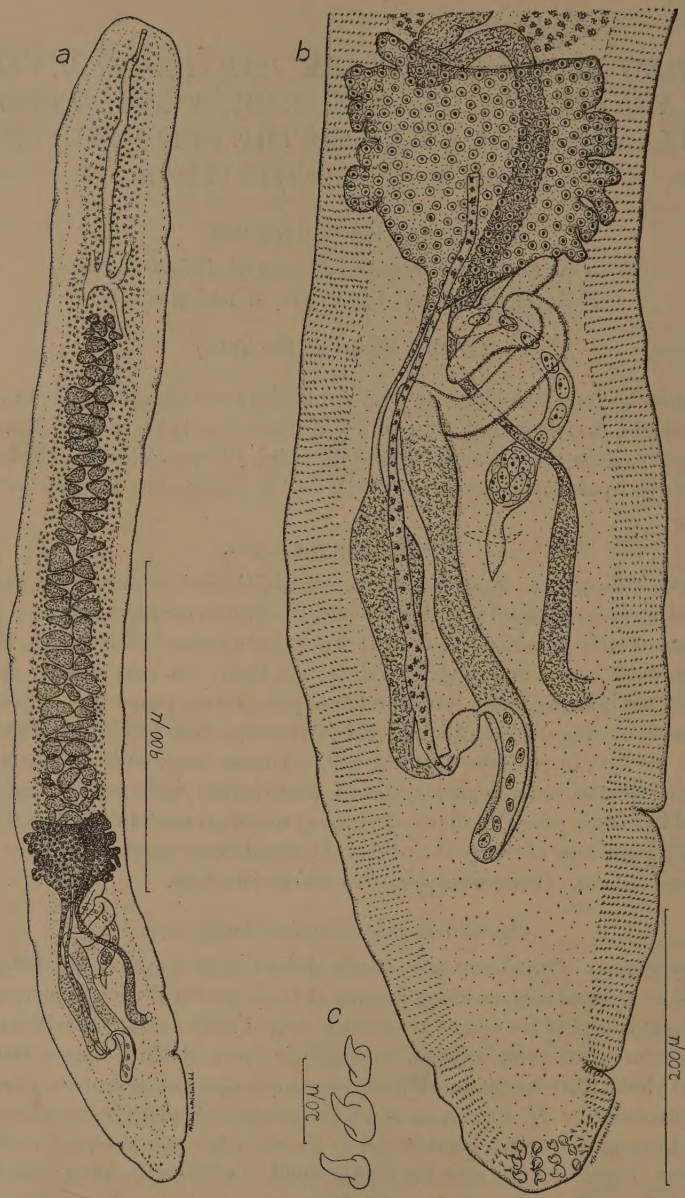

Fig. 1. *Paradeontacylix sanguinicoloides* n.g., n.sp. *a*, complete specimen, ventral aspect, spines not shown; *b*, posterior third of body showing arrangement of spines; *c*, three spines from posterior end of body, greatly enlarged.

diverticula, two extending anteriorly and two posteriorly, longest measuring approximately 147μ. Testes ovoid, approximately sixty in number, averaging about 60μ long by 90μ wide, arranged in two irregular rows between fork of crura and ovarian zone; zone of testes 1·342 mm. long, field 185μ wide. Vas deferens well developed, apparently serving as seminal vesicle, leading from testes to male genital pore; cirrus not observed; male genital pore dorsal, 457μ from posterior end and about 61μ from left margin of body. Ovary somewhat shield-shaped, 213μ long by 244μ wide, with lobed or fissured margins. Oviduct 457μ long, originating from apex of shield, and leading to ootype; posterior half of oviduct dilated, serving as a seminal receptacle. Vitellaria extensive, occupying most of available space from near anterior end of body to ovarian zone; vitelline duct well developed, median, extending between oviduct and uterus, from ovarian zone to ootype. Two indistinct tubes, lateral to the testes, apparently the paired vitelline ducts, observed, but not traceable posteriorly to union with unpaired median duct. Ootype 45μ by 30μ, 397μ from posterior end of body; Laurer's canal not observed. Uterus posterior to ovary, extending from ootype posteriorly about 120μ, then anteriorly as far as ovary, making a few coils, and then descending to female genital pore; proximal portion of uterus filled with spermatozoa; terminal portion dilated, containing several eggs. Female genital pore dorsal, 152μ antero-mesal of male genital pore. Eggs oval, 17·8μ by 11·5μ, with thin colourless shells.

Type specimen. U.S. N. M. Helm. Coll. No. 34329.

Habitat. Blood vessels of gills of *Seriola lalandi*, Atlantic Ocean off Miami, Florida.

Remarks. On the basis of the long coiled uterus and the postovarian genital pore, the new genus *Paradeontacylix* is more closely related to *Deontacylix* Linton 1910, than to any other genus. *Paradeontacylix* may be separated from *Sanguinicola* Plehn 1905, and *Janickia* Rašín 1929, on the length of the uterus, which in these is very short and without coils; the species of these two genera are all from fresh-water fishes. *Janickia* is regarded by the writer as a synonym of *Sanguinicola*, since, according to Rašín (1929), it does not differ from the genus *Sanguinicola* except in shape and size of the egg, ootype, and metraterm; the species *Janickia volgensis* Rašín 1929, therefore, becomes *Sanguinicola volgensis* (Rašín 1929) n.comb.

Paradeontacylix sanguinicoloides differs from *Aporocotyle simplex* Odhner 1900, also from a marine fish, in that the uterus and genital pores are postovarial, while in *A. simplex* the uterus and genital pore are preovarial. *Paradeontacylix sanguinicoloides* has many points in common with *Aporocotyle odhneri* Layman 1930, the arrangement of the testes, and location of ovary and uterus being similar in both species, and on the basis of this similarity the writer believes that *A. odhneri* should be removed from the genus *Aporocotyle*; it is certainly not congeneric with *A. simplex* Odhner 1900. Although Layman's (1930) description is not complete in certain details, the writer believes that *A. odhneri* has sufficient characters in common with *Paradeontacylix sanguini-*

coloides to justify regarding the two species as congeneric, the name, *Aporocotyle odhneri*, therefore, becoming *Paradeontacylix odhneri* (Layman 1930) n.comb.

Family Aporocotylidae

Odhner (1912) proposed the family Aporocotylidae for the *Aporocotyle-Sanguinicola* group of blood flukes of fishes. The confusion in this family is probably not equalled in any other group of parasites. Odhner (1900), in describing the first species, *Aporocotyle simplex*, regarded it as an ectoparasite of the gills, but later (Odhner, 1911) pointed out that it was a blood parasite. Plehn (1905), in describing the two original species of *Sanguinicola*, *S. armata* and *S. inermis*, regarded them as ectoparasitic turbellarians. In a subsequent paper, Plehn (1908) regarded these species as haematobic monozoic cestodes. Odhner (1911) was apparently the first to publish on *Sanguinicola* as a digenetic blood fluke. He also pointed out at this time the relationship of *Aporocotyle* and *Deontacylix* to *Sanguinicola*. Linton (1910), in describing *Deontacylix ovalis*, was not, apparently, aware that he was describing a blood fluke, as he made no mention of it as having been found in the circulatory system.

Odhner (1924), in his "Remarks on *Sanguinicola*," named a third species of *Sanguinicola*, *S. chalmersi*, which had been reported by Woodland (1923) from the blood of Sudan siluroids. Ejsmont (1926), in a rather comprehensive study of the species of the genus *Sanguinicola*, described a fourth species, *S. intermedia*. Rašín (1929) described *Janickia volgensis*, which belongs to the genus *Sanguinicola* as pointed out earlier in this paper. Van Cleave and Mueller (1932) have described the only American species of *Sanguinicola*, *S. occidentalis*.

The above species of the family Aporocotylidae may be separated by the aid of the following keys:

Key to Genera of Family Aporocotylidae

1. Uterus short, without coils, consisting only of metraterm; parasites of fresh-water fishes ... ... **Sanguinicola** Plehn 1905
   Uterus long, coiled; parasites of marine fishes **2**
2. Genital pore preovarial ... ... **Aporocotyle** Odhner 1900
   Genital pore postovarial **3**
3. Uterus both preovarial and postovarial; ovary lateral ... **Deontacylix** Linton 1910
   Uterus postovarial; ovary median ... ... **Paradeontacylix** n.gen.

The genus *Aporocotyle* has only the one species, *simplex* Odhner 1900; the genus *Deontacylix* also has but a single species, *D. ovalis* Linton 1910. A key is given here for the species of each of the other two genera.

Key to species of genus Sanguinicola

1. With paired vitelline ducts; cuticular spines in irregular arrangement ... ... **S. occidentalis** Van Cleave and Mueller 1932
   With unpaired vitelline duct; cuticular spines in a single marginal series, or absent ... 2
2. Body with no cuticular spines, at most only setae present ... **S. inermis** Plehn 1905
   Body with a single row of cuticular spines 3
3. Cuticle of body with fine setae ... ... **S. intermedia** Ejsmont 1926
   Cuticle of body without fine setae 4
4. Intestine not lobulated; 6 to 7 pairs of testes ... ... **S. chalmersi** Odhner 1924
   Intestine lobulated; 10 or 20 pairs of testes 5
5. Ten pairs of testes ... ... **S. armata** Plehn 1905
   Twenty pairs of testes ... ... **S. volgensis** (Rašín 1929)

Key to species of genus Paradeontacylix

Ovary shield-shaped, situated one-third of body length from posterior end ... ... **P. sanguinicoloides** n.sp.

Ovary arcuate, situated one-ninth of body length from posterior end ... ... **P. odhneri** (Layman 1930)

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THE LIFE HISTORY OF *LEIDYNEMA APPENDICULATA* (LEIDY), A NEMATODE OF COCKROACHES[1]

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(With Plate XXIII and 10 Text-figures)

Introduction

Entozoa from insects, including a description of the nematode, *Oxyuris diesingi*, from the cockroach, *Blatta orientalis*, were first reported by Hammerschmidt in 1838. In 1847 he described another nematode, *Oxyuris blattae orientalis*, from the same host. Both forms were described by Leidy (1850), who gave the name *Aorurus streptostoma gracile* to the first and *A. thelastomus appendiculatum* to the second. Diesing (1851) described *Oxyuris diesingi* under the name of *Anguillula macrurae.*

The contributions of Bütschli (1871), Galeb (1878), Magalhães (1900), Pessoa and Correa (1926), Schwenk (1926) and Walton (1927) deal primarily with descriptions of new species or with classification and redescriptions of old forms. Chitwood (1932), dealing with the taxonomy of nematode parasites in Blattidae, transferred *Oxyuris diesingi* to the new genus *Hammerschmidtiella* and placed both of these species in the family Thelastomidae Travassos. Since Hammerschmidt's *Oxyuris blattae orientalis* appears to be *O. diesingi* redescribed, it becomes a synonym of both *Hammerschmidtiella diesingi* and *Leidynema appendiculata* (Leidy, 1850) Chitwood, 1932.

The intestinal diverticulum is used as a family character by Yorke and Maplestone (1926), Sprehn (1932) and other helminthologists. Chitwood employs it as a generic character. On the basis of this character *L. appendiculata* should be transferred to the family Cruzidae Travassos, 1917, or to a new family.

As most of the work dealing with this group of nematodes has been taxonomic or anatomical the emphasis, in this paper, is placed on the life history of these oxyurids, which are of common occurrence in the American cockroach (*Periplaneta americana*) at Manhattan, Kansas. Although both of the oxyurids were utilised in this investigation more consideration has been given to *Leidynema appendiculata.*

Incidence of oxyurids in cockroaches at Manhattan, Kansas

These parasitic nematodes are found in the hindgut of the cockroach; they are most numerous in the region of the Malpighian tubules.

The data for determining the incidence of oxyurid infestation were based on the examination of *Periplaneta americana* taken under natural conditions.

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Most of the cockroaches were killed immediately after capture, but some were confined for a few days before examination. As shown in Table I, male, female, young and some mature *P. americana* whose sexes were not recorded were examined for nematodes. In all 259 cockroaches were examined and of these 222 were infested with either one or both species of the oxyurids. Of the 222 infested roaches 40·1 per cent. harboured *Leidynema appendiculata*, 21·1 per cent. *Hammerschmidtiella diesingi*, and 28·8 per cent. of them had both species of worms. It was found that hosts collected from the same habitat, such as a room or box, usually had a predominance of the same species of parasites. The results also show that 79·5 per cent. of the males, 87·5 per cent. of the females and 94·2 per cent. of the immature roaches harboured these parasites. There was no apparent difference between the males and females which would account for the higher infestation in the latter. The difference in infestation of the young and the mature roaches may have been due to age resistance of the adults. There also appears to be some natural resistance to the parasites, as about 15 per cent. of the cockroaches in the same habitat were not infested.

Table I. *Incidence of cockroach oxyurids at Manhattan, Kansas.*

	Total no.	No. infested	Infested %	No. with *L. appendiculata*	No. with *H. diesingi*	No. with *H. diesingi* and *L. appendiculata*
Males	98	78	79·5	36	14	21
Females	80	70	87·5	22	17	28
Immature	52	49	94·2	20	13	9
Adults, sex not recorded	29	25	84·1	12	3	6
Total	259	222	86·3	90	47	64
Infested roaches having one or both species of oxyurids (%)				40·1	21·1	28·8

In determining the sizes of the infestations only the mature nematodes were recorded. The range of infestation was from 1 to 36 with an average of 3·8 worms per male, 5·1 worms per female, and 2·7 worms per immature roach. In the male and female *Periplaneta americana* there appears to be a direct correlation between incidence and size of infestation, but this does not hold for the immature roaches in which the incidence of oxyurids was comparatively high and the size of infestation low. Ackert and Wadley (1921) obtained similar results from nematodes parasitic in the cricket, *Gryllus assimilis* Fab. They found that 85 per cent. of the adults, 70 per cent. of the males, and 90 per cent. of the female crickets were infested and that the females contained larger numbers of the parasites.

Host-parasite relationship

Periplaneta americana apparently is not markedly affected by these oxyurid parasites. Heavily infested cockroaches were maintained in captivity for more than a year without apparent injury. Transverse sections of infested roach intestines showed no evidence of tissue destruction. In fact, all observations seemed to indicate that the health, fertility and activity of the heavily

infested cockroaches were comparable with those of the non-parasitised specimens.

Morphology of the adult worms

A description of the general structure of *Hammerschmidtiella diesingi* and *Leidynema appendiculata* was given by Bütschli (1871). The illustrations by Galeb (1878) are very diagrammatic but they give the distinguishing features of the two nematodes. The typical female *Hammerschmidtiella diesingi* is characterised by an anterior oesophageal pseudobulb, absence of an intestinal diverticulum, posterior intestine without a loop, and vulva in anterior part of body. On the other hand, the female *Leidynema appendiculata* has a cylindrical anterior oesophagus, presence of intestinal diverticulum, posterior intestine with a single loop, and vulva located near the middle of the body.

The *Hammerschmidtiella diesingi* male has an anterior oesophageal pseudobulb, and tail obliquely truncate with a long tapering thickened portion attached dorsally. The *Leidynema appendiculata* male has a prebulbar swelling in the oesophagus and truncate tail with a very small dorsally attached end-piece. The distinction between the males of these species is no doubt correct, for on several occasions copulation was observed in both *Hammerschmidtiella diesingi* and *Leidynema appendiculata*.

Concerning this phenomenon, an interesting case was observed. In *Hammerschmidtiella diesingi*, four males had been attracted to a female. Although there was no copulation, the four male specimens adhered to the body of the female, indicating the presence of some adhesive substance. Whether or not this facilitates copulation is unknown. In the cases of copulation seen, the curved posterior end of the male was nearly at right angles to the female body, the anterior end extending out obliquely from that of the female. The positions were similar for *Leidynema appendiculata*.

Leidynema appendiculata (Leidy, 1850) Chitwood, 1932

Small, rather thick-bodied nematodes with semi-transparent cuticula. The following measurements were made on fixed specimens.

Males[1]. Length: 0·525–0·850, average 0·749. Width: 0·045–0·087, average 0·068. Cuticular striations: length 0·003. Oesophagus: length 0·133–0·185, average 0·167. Anterior oesophagus: length 0·08–0·125, average 0·109; width 0·015–0·02, average 0·017. Isthmus: length 0·017–0·02, average 0·018; width 0·009–0·12; average 0·01. Bulb: length 0·034–0·04, average 0·037; width 0·027–0·036, average 0·031. Nerve ring: 0·075–0·105 from anterior end of body, average 0·102. Excretory pore: 0·2–0·22 from anterior end of body, average 0·21. Anus: 0·01–0·15 from tip of tail, average 0·013. Spicule: 0·026–0·035, average 0·029.

The general morphology of the male nematode is shown in Fig. 1 (Pl. XXIII). The body is usually bent and has a characteristically curved posterior end.

[1] All measurements in mm.

The cuticular striations are more prominent at the anterior end. The posterior end of the body is studded with a pair of large preanal, a pair of small postanal, a pair of indistinct subdorsal papillae, and a very minute pointed dorsal end piece. The oesophageal bulb is very conspicuous. The intestine is simple without a diverticulum or a loop. In the posterior half of the body is the reproductive system. The testis is a compact structure near the middle of the body. It opens directly into the sperm duct which appears to be surrounded throughout by large glandular cells. The short ejaculatory duct opens into the cloaca. A single spicule is present.

Females[1]. Length: 2·27–4·025, average 3·25. Width: 0·238–0·35, average 0·325. Oesophagus: length 0·435–0·497, average 0·463. Anterior oesophagus: width 0·045–0·085, average 0·063. Isthmus: length 0·021–0·045, average 0·032; width 0·035–0·04, average 0·036. Bulb: length 0·09–0·106, average 0·098; width 0·1–0·148, average 0·111. Nerve ring: 0·135–0·195 from anterior end of body, average 0·165. Excretory pore: 0·505–0·6 from anterior end of body, average 0·531. Intestinal diverticulum: length 0·45–0·75, average 0·595. Anus: 0·42–0·825 from tip of tail, average 0·675. Vulva: 1·05–1·985 from anterior end of body, average 1·519. Eggs: length 0·106–0·112, average 0·108; width 0·045–0·048, average 0·46.

The general morphology of the female nematode is shown in Fig. 2 (Pl. XXIII). Like other oxyurids the stout body terminates posteriorly in a long, tapering tail. The cuticle is closely annulated from the anterior end to the tail. Lateral alae extend posteriorly from the region of the oesophageal bulb, terminating in spine-like projections. The mouth as shown by Chitwood is surrounded by eight large submedian labio-papillae. The oesophagus consists of a long cylindrical anterior portion which connects posteriorly by a narrow isthmus with the oesophageal bulb. From the anterior part of the intestine a large diverticulum, varying considerably in size, emerges laterally and posteriorly. Its function has not been determined. In the posterior half of the worm the intestine forms a distinct loop. The nerve ring is usually located in the region of the prebulbar swelling; while the excretory pore is posterior to the oesophageal bulb.

The essential parts of the female reproductive organs are illustrated in Figs. 2 and 3 (Pl. XXIII). The vulva of *Leidynema appendiculata* is located slightly posterior to the middle of the body. From the genital orifice the vagina extends dorsally to the opposite side of the body where it joins the opposed uteri. Ordinarily the anterior uterine branch extends forward to the distal end of the intestinal diverticulum, and the posterior branch to slightly beyond the intestinal loop. The oviducts, leading from the uteri, extend for varying distances in the same direction as their respective uteri, then each duct reverts and follows an irregular course to the opposite end of the body cavity. From there the ducts make another complete turn, and each branch then continues its new course for a distance which varies in different specimens, until it unites

[1] All measurements in mm.

with its respective ovary. The anterior ovary extends from the region of the intestinal diverticulum to the posterior third of the body, while the other ovary extends from the extreme posterior end of the body to the anterior third of the worm.

Experimental data and observations

Technique of slide preparation

The cuticular and muscular layers of many of these nematodes are so transparent that most of the structures can be distinguished without the use of reagents. Of the fixatives tried Carnoy and Lebrun's fluid fixed with the least distortion, but it produced a 10 per cent. shrinkage of the worms. In general, stains in alcoholic solutions such as haemacalcium, paracarmine, and Grenacher's carmine proved to be more satisfactory than those in aqueous solvents.

Rearing cockroaches

The stock specimens of cockroaches were kept in open fruit jars and in one-gallon tin cans. To prevent the escape of the cockroaches, the walls of the containers were thinly coated with stale butter. The food requirements of the roaches were very simple; a few drops of water and bits of table scraps constituted a weekly ration. When so supplied with food, there was little or no cannibalism. Under these conditions large numbers of cockroaches were maintained for more than a year with very low mortality. To rear uninfested roaches, oöthecae were collected from the natural habitats of the insects and placed in clean fruit jars where the young nymphs hatched in about 6 months. Three to four additional months were required for growth to sizes suitable for experimental use.

Culturing the oxyurid eggs

The best egg cultures were prepared by placing the female worms in a one-third normal Ringer's solution on glass slides. Here, in about 12 hours, they laid practically all of their eggs. The subsequent removal of the female worms left débris-free cultures for incubation and study. To prevent drying the culture slides were placed in Petri dishes containing a few drops of water. Storing large numbers of cultures was facilitated by stacking the slides in a moist chamber.

While a few eggs of ascarids usually hatch in the ordinary culture media, those of these oxyurids failed to do so. Although the eggs were cultured in such media as Locke's solution, distilled water, peptone, dilute formalin and faecal extracts, the mature embryonated eggs (Text-fig. 4) did not hatch in the media. Failing with these culture media, tests were made by placing embryonated eggs in artificial digestive fluids and concentrated filtered extracts from the foregut and other parts of the cockroach's alimentary canal. These and other tests were conducted for from 4 to 50 days, but in no instance was a free larva seen in any of the media.

Viability of the nematode eggs

Eggs of these nematodes when kept moist at room temperatures and in darkness or greatly subdued light developed normally and remained viable for considerable periods of time. But when such eggs were exposed to strong, indirect sunlight development ceased, and if the exposure continued the eggs died. Fifteen-minute exposures of eggs to the rays of a 400-watt projection lamp passed through a water bath killed most of the embryos in the early stages of cleavage. The heat from this lamp, 40° C., was not sufficient to kill the eggs. That light may be lethal has been shown by Caldwell and Caldwell (1928) and Otto (1929), who found it to be an important factor in the destruction of ascarid eggs. The experiments by Nolf (1932) show that wave-lengths of light ranging from 180 to 315 mμ are highly fatal to ascarid ova.

Concerning oxygen requirements, there appears to be nothing unusual in these oxyurid eggs. Those in water mounts under cover-slips showed arrested development, but continued to develop when additional air was admitted. The fertilised eggs in the bodies of dead nematodes did not divide until decomposition of the worms occurred. Of the eggs in various developmental stages subjected to varying degrees of desiccation, those in the early stages were much more readily killed than were the embryonated eggs. From these observations it may be seen that the habits of cockroaches in seeking out dark corners and crevices in relatively moist atmospheres are conducive to the viability and development of these oxyurid eggs after deposition.

Transmission of the nematodes

It was assumed by Bütschli (1871) that transmission of these nematodes is direct. To the writers' knowledge no previous transmission experiments have been made with parasitic nematodes from the Blattidae. A series of experiments was carried out to determine (1) the stage at which the eggs become infective, and (2) the means of transmission. The oxyurid eggs were incubated to the embryonated stage and then fed with food or water to uninfested cockroaches. The results of these experiments are shown in Table II.

To three roaches (Group A) were fed *Leidynema appendiculata* eggs in various stages of development. One cockroach examined 4 days later was infested with six worms. While the other two roaches which were killed on the 5th and 6th days respectively were without worms, the results of this experiment indicate that transmission is direct.

In carrying out the remaining transmission experiments, a distinction was made in the kind of embryonated eggs administered. Those eggs containing nearly mature motile embryos (Text-fig. 2) are hereinafter referred to as the active embryonated stage, while the eggs containing mature non-motile embryos are called the resting embryonated stage (Text-figs. 3 and 4).

To four uninfested roaches (Group B) was given faecal matter from infested roaches that contained oxyurid eggs in the resting embryonated stage.

In from 3 to 7 days, each of these roaches was examined with the result that three of the four contained worms (Table II). These results indicate that the resting embryonated stage is infective.

In the next experiment (Group C) eggs of *L. appendiculata* in the active embryonated stage were given to seven uninfested cockroaches. Five days later, the examination of a roach proved negative. On the 7th day after feeding, the examination of a second roach proved negative. At this time, the remaining five roaches were given a second feeding of active embryonated eggs

Table II. *Showing results of transmission experiments.*

Cockroaches		Nematodes			
Group	No.	Species	Kinds of embryonated eggs fed	Days parasitism before examination	Worms found
A	1	*L. appendiculata*	Active and resting	4	6
	2	,,	,,	5	0
	3	,,	,,	6	0
B	1	*L. appendiculata* and *H. diesingi*	Resting	3	1
	2	,,	,,	4	4
	3	,,	,,	6	0
	4	,,	,,	7	4
C	1	*L. appendiculata*	Active	5	0
	2	,,	,,	7	0
	3	,,	,,*	21	0
	4	,,	,,*	21	0
	5	,,	,,*	21	0
	6	,,	,,*	24	0
	7	,,	,,*	24	1
D	1	*L. appendiculata*	Resting	25	0
	2	,,	,,	25	1
	3	,,	,,	25	5
E	1	*H. diesingi*	Active	6	0
	2	,,	,,	6	0
	3	,,	,,	6	0
	4	,,	,,	6	0
	5	,,	,,	6	0
	6	,,	,,	7	0
F	1	*H. diesingi*	Resting†	5	7
	2	,,	,,†	6	4
	3	,,	,,†	6	0
	4	,,	,,†	6	0

* A second culture of active embryonated eggs was fed 7 days later.

† Eggs from culture 2 months old.

of *L. appendiculata*. In order to give time for development, the next examination was not made until 14 days after the last feeding of embryonated eggs. On that date, three of the five remaining roaches were examined but all were negative. On the 17th day after the second feeding, the sixth cockroach was examined. It likewise was negative. The examination of the last roach of this experiment on the 17th day (24 days after the first feeding) contained one worm. The results of this experiment indicate that the active embryonated stage is non-infective. The presence of the one worm can be accounted for on the basis of a resting embryonated stage having been overlooked in the experimental feeding.

The fourth test of this series consisted of feeding eggs of the oxyurid *L. appendiculata* in the resting embryonated stage to three uninfested cockroaches (Group D). These roaches were examined on the 25th day after feeding, with the result that one was negative and the other two positive, one having one worm, the other having five. These results give further evidence that the infective eggs of this nematode are those in the resting embryonated stage.

To test the active embryonated stage of the oxyurid *Hammerschmidtiella diesingi*, six uninfested cockroaches (Group E) were given eggs in that stage. The examination of this group of cockroaches on the sixth and seventh days after feeding showed that all of the roaches were negative, giving further evidence that the active embryonated stage is non-infective. To test the resting embryonated stage of *H. diesingi*, eggs in this stage taken from a two-month old culture were given to four uninfested cockroaches (Group F). The examinations made on the fifth and sixth days afterward showed that two of the cockroaches were infested with seven and four worms respectively, and that the other two roaches were negative.

From the results of this and the other experiments, the writers conclude that the infective stage is the resting embryonated stage and that transmission of the oxyurids *Leidynema appendiculata* and *Hammerschmidtiella diesingi* from one cockroach to another is direct. The small infestations were doubtless due to the limited number of embryonated eggs fed to the cockroaches. For heavy infestations and a higher incidence repeated feedings of large numbers of eggs probably are necessary. Under natural conditions, the cockroaches constantly feed on infected food or faecal matter which would account for the somewhat heavier natural infestations and higher incidence shown in Table I.

Life cycle

Development of the egg

The general structure of the female reproductive system of *Leidynema appendiculata* is much like that found in most nematodes. The germinal zone containing nuclei is located in the distal portion of the ovary (Pl. XXIII, fig. 2). The nuclei migrate away from the distal end where the ova are formed. In the developmental area the ova are arranged in layers much like piles of discs. As they approach the proximal end they become surrounded with yolk material and pass from the ovaries into the oviducts where they appear as irregular masses, but midway between the ovary and uterus they assume a more characteristic shape. The shell glands appear to be near the regions where the oviducts lead into the uteri. Just where fertilisation takes place was not determined. Eggs in the uteri are usually unsegmented; however, in a few living specimens intra-uterine eggs were developed slightly beyond the two-cell stage. In culture media the gravid females often underwent a period of rapid egg laying until the uteri were empty, while other females retained their eggs several days. Eggs may be discharged as rapidly as one every 5 sec. In one

instance a worm laid sixty eggs in 90 min. The eggs are oval, elongate and flattened on one side. They are rather thin shelled and in different specimens vary considerably in length and width. Magalhães (1900) states that the shell of *Thelastoma bulhoesi* (Magalhães, 1900) Travassos, a related species, is composed of two parts, of which the internal one is very thin and becomes visible only after the use of reagents.

At the time of extrusion the eggs are undeveloped or in the two to four-celled stages.

The morula with small blastomeres is followed by a period of embryonic growth which results in the prevermiform stage (Text-fig. 1). As indicated in the figure, this flattened embryo has a tail bud at the posterior extremity. The individual cell structure is no longer obvious. Growth now occurs both anteriorly and posteriorly until a motile tadpole-like embryo is formed (Text-fig. 2). This embryo, which has been designated as the active embryonated stage, has a small pointed tail and a comparatively large blunt anterior end. The digestive tract consists of a simple undifferentiated tube. This young form contracts its body and squirms about, but it retains its same relative position within the egg. As the development of the embryo progresses to the next stage (Text-fig. 3) the wriggling decreases and then ceases. The young form is contracted and non-motile, the tail being reduced to a mere stub. The anterior oesophagus and oesophageal bulb are visible. In the last stage before hatching the embryo resembles the stage just described but shows a higher degree of development. It is much more contracted and rounded. There is evidence of a striated cuticle. The characteristic oesophageal bulb can be seen distinctly in the posterior half of the body. The anterior oesophagus is readily recognised. This non-motile form is designated as the resting embryonated stage, which doubtless is the infective stage.

When incubated at 37° C., the development of the eggs is rather rapid. The undeveloped eggs often reach the two-celled stage in less than an hour. By the end of 12 hours development has progressed to the morula with small blastomeres, and in 12 more hours the active embryonated stage is reached (Text-fig. 2). After 3 to 7 days, during which the active embryo gradually loses its motility and develops the digestive tract, the infective or resting embryonated stage (Text-figs. 3, 4) is reached.

Hatching of eggs

Since the natural mode of transmission of these nematodes is by ingestion of infective eggs, hatching must take place somewhere in the alimentary canal of the cockroach. In order to determine where hatching occurs the crop, midgut and hindgut of each cockroach examined were searched separately for eggs and larvae. In a few instances eggs with mature embryos were found in the crop but never in the midgut. The hindgut often contained empty egg shells and eggs, but the latter were always in the early stages of development. Young larvae were present in the posterior part of the midgut and in the hind-

gut but absent in the rest of the digestive tract. From these observations it appears that the eggs hatch in the posterior part of the midgut.

Growth and development of the larvae

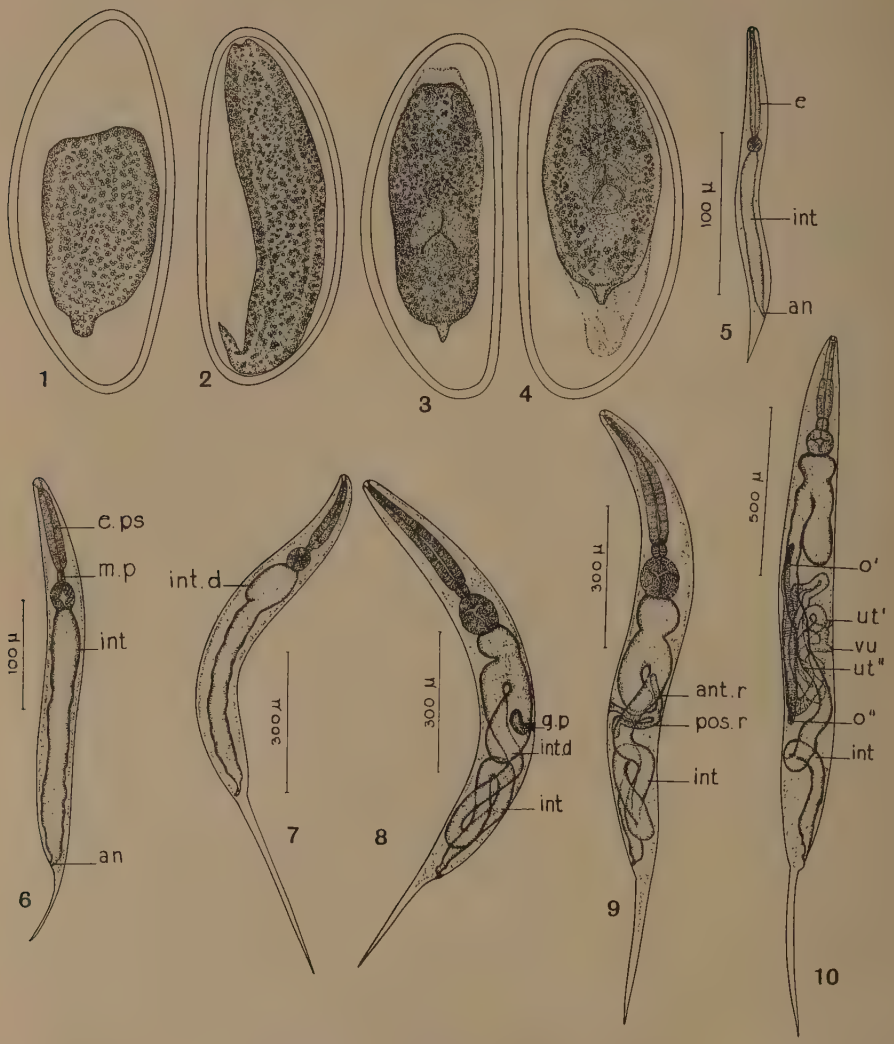
The youngest larvae found measured from 150 to 170μ in length. At this stage it was impossible to distinguish *Leidynema appendiculata* from *Hammerschmidtiella diesingi*. In a slightly older larva (Text-fig. 5) which measured 208μ in length the oesophagus resembles that of a mature *Leidynema appendiculata*; the intestine is a simple tube. At the corresponding stage the larvae of *Hammerschmidtiella diesingi* have an oesophageal pseudobulb anterior to the true bulb. The entire cuticle of both larval forms is annulated much like the adults.

A larva 430μ long (Text-fig. 6) was recovered 12 days after parasitising. In this specimen the oesophagus is divided into distinct anterior and posterior parts connected by a narrow isthmus (midpiece). The intestinal walls were irregular and the anterior portion had begun to enlarge. The larva illustrated in Text-fig. 7, although over a millimetre long, was but slightly more developed than the preceding one. The intestinal diverticulum appears to develop as a pouch from the enlarged anterior intestine. Many of the larvae, as the two just described, show also great variations in the length of the tail.

Specimens of *Leidynema appendiculata* larvae which represent stages in the development between those shown in Text-figs. 7 and 8 are so opaque that in spite of treatment the internal anatomy could not be clearly determined. The opacity may be due to the two-layered cuticula prior to moulting. All of these young sausage-like forms are comparatively much shorter and thicker than the other larvae. However, in these specimens the intestine appears to be thick-walled, irregular and coiled. The oesophagus is typical and the genital primordium is a small mass of heavily stained cells.

The immature worm at the next stage (Text-fig. 8) bears much resemblance to the adults. It is characterised by a typical oesophagus, a conspicuous intestinal diverticulum and a greatly coiled posterior intestine. The genital primordium, a small protuberance extending dorsally from the genital orifice, is located in the region of the diverticulum. A slightly later stage (Text-fig. 9) shows a little modification in most of the essential structures. The body is somewhat longer and the posterior intestine more distended and less convoluted; the most marked change is in the reproductive organs. Because of the parasite's growth the genital primordium is posterior to the intestinal diverticulum. The reproductive anlage extends from the vulva dorsally to the opposite side of the body where it forks into short branches, one anterior, the other posterior. The worms shown in Text-figs. 8, 9 and 10 measure respectively 1·2, 1·3, and 2 mm. in length.

The immature female (Text-fig. 10) resembles closely the two forms just described. The positions of its internal organs do not differ significantly from those of the adult worms. In most but not all of the older immature and fully



Text-figs. 1–10. *Leidynema appendiculata.*

1. Prevermiform stage. 2. Active embryonated stage. 3, 4. Resting embryonated stage. 5. An early stage larva. 6. Twelve-day old female. 7. Young female showing enlargement of cardia. 8. Female showing intestinal diverticulum, coiled intestine and genital primordium. 9. Female showing a stage in development of the reproductive system. 10. Young female with immature reproductive system.

For letterings see the explanation of Plate XXIII.

developed worms the oesophagus is reduced in comparative length and size. The intestine of this young worm has a single loop in the posterior part of the body. The reproductive organs consist of a vulva, a short vagina, an immature anterior and posterior uterus. Each of these uteri is joined with an undeveloped ovary. At this stage the oviducts cannot be distinguished from the uteri. In the mature worm (Pl. XXIII, fig. 2) the organs of the reproductive system are larger and distributed throughout the body cavity posterior to the oesophagus.

Summary

1. Of 259 cockroaches (*Periplaneta americana*) collected at Manhattan, Kansas, 86·3 per cent. were infested with one or two species of oxyurids, *Leidynema appendiculata* and *Hammerschmidtiella diesingi*. The highest incidence was among the immature roaches, of which 94·2 per cent. were infested; next was in the adult females with 87·5 per cent. infested; and lastly among the mature males with 79·5 per cent. parasitised. The female roaches carried the heaviest infestations and the young *Periplaneta americana* the lightest.

2. Uninfested cockroaches were obtained by rearing the young from oöthecae.

3. Oxyurid eggs in moist chambers remained viable for at least two months. Eggs of all stages when exposed to direct strong artificial or natural light for 15 min. failed to continue development.

4. Fertilised eggs incubated at 37° C. in dilute Locke's solution develop to the active embryonated stage in 20–36 hours; in 4–7 days they develop to the resting embryonated stage.

5. Regardless of the media utilised eggs failed to hatch *in vitro*.

6. Feeding experiments proved that transmission is direct. Eggs in the resting embryonated stage are infective; those in the active embryonated stage do not appear to be infective.

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LETTERING TO FIGURES

a.e.	anterior oesophagus	*o′*	anterior ovary
an	anus	*o″*	posterior ovary
ann	annulations	*ov′*	anterior oviduct
ant.r.	anterior branch reproductive system	*ov″*	posterior oviduct
ca	cardia	*po.an.p.*	postanal papilla
cl	cloaca	*pos.r.*	posterior branch reproductive system
e	oesophagus	*pr.an.p.*	preanal papilla
e.b.	oesophageal bulb	*r*	rectum
eg	egg	*s.d.*	sperm duct
ej.d.	ejaculatory duct	*sd.p.*	subdorsal papilla
e.p.	end piece	*sp*	spicule
e.ps.	oesophageal pseudobulb	*spr*	spermatozoa
g.c.	glandular cells	*ta*	tail
g.p.	genital primordium	*te*	testis
int	intestine	*ut′*	anterior uterus
int.d.	intestinal diverticulum	*ut″*	posterior uterus
m	mouth	*va*	vagina
m.p.	midpiece	*vu*	vulva
n.r.	nerve ring		

EXPLANATION OF PLATE XXIII[1]

Fig. 1. Mature *Leidynema appendiculata* male.

Fig. 2. Mature *L. appendiculata* female.

Fig. 3. Diagrammatic drawing showing reproductive system of female *L. appendiculata.*

[1] Indebtedness is acknowledged to Marjorie Prickett Dobrovolny for making most of the drawings.

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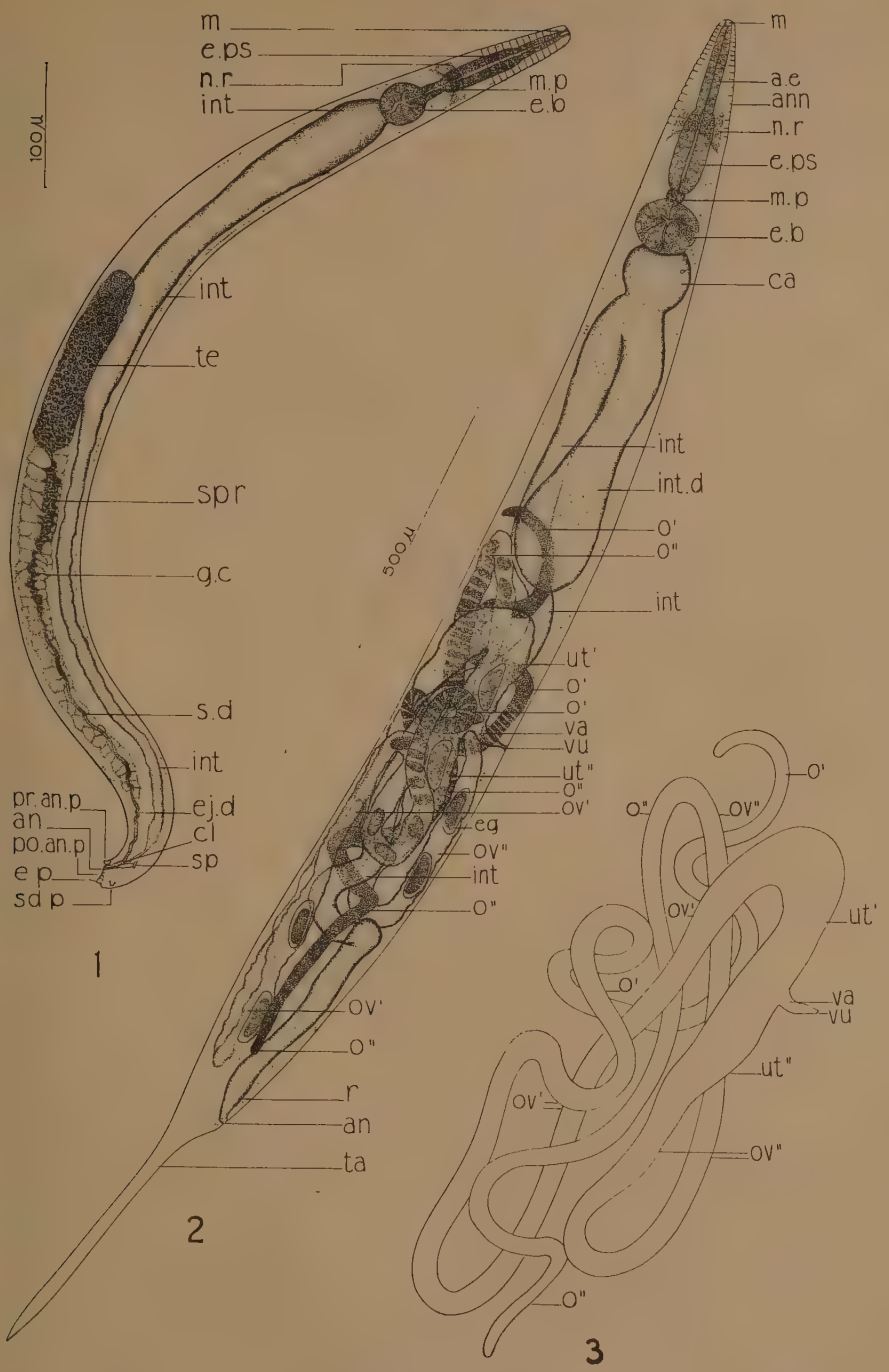
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ON A COLLECTION OF PARASITIC WORMS FROM MALAY

I. NEMATODES (SUPERFAMILIES ASCAROIDEA AND OXYUROIDEA)

By V. C. ROBINSON

Molteno Institute for Research in Parasitology, Cambridge

(With Plates XXIV–XXVI, containing Figs. 1–26)

The present paper deals with a collection of parasites from Malay, kindly sent to Prof. Keilin at the Molteno Institute by G. B. Purvis, F.R.C.V.S., to whom the writer wishes to express his appreciation of the painstaking and scientific way in which the collection was carried out. The specimens were all very carefully sorted and clearly labelled, and the majority extremely well preserved.

The Nematodes dealt with in the following account are the Ascaroidea from a python, *Python reticulatus* Schneid., and the Oxyuroidea from a flying lemur, *Galeopithecus volans peninsulae* Thos.

ASCAROIDEA

ASCARIDAE

Ascarinae

Genus **Ophidascaris**

O. filaria Duj. 1845.

About seventeen specimens of this worm were collected from the intestine of a python at Alor Star. Baylis (1920) has given a useful revision of the Ascarids in snakes and, as the present specimens differ a little from his account of the species, a full description is added.

The worms are slender, much attenuated at the anterior end and thicker at the other. The chief measurements are as follows: Female 145 mm., male 120 mm. Average maximum breadth about 2 mm. Head end 0·35 mm. across. The grooves at the bases of the lips are well marked, those on the dorsal lip approximating very close to one another. Lips broader than long (dorsal lip 0·23 mm. by 0·15 mm.). The outline of the dorsal lip differs from that in Baylis' figure in that the anterior edge is not indented, but slightly concave. There are two simple papillae on this lip, situated near the antero-lateral corners of the pulp. Each ventral lip, as far as could be made out, has one large simple papilla. The oesophagus is 5·5 mm. long. The tail end in both sexes is rounded, with no terminal spike. The eggs are nearly spherical, 0·10 mm. in diameter and have a thin, unornamented shell.

Male: The spicules are shaped like scimitars, with a thickened dorsal shaft

and a thin blade or ala. Their free extremities are rounded. The spicules are slightly unequal, the left one being 2·2 mm. long and 0·1 mm. broad, the right one 1·75 mm. long and 0·08 mm. broad. The tail is 0·45 mm. long. There is no bursa. The postanal papillae are arranged as described by Baylis and there are forty pairs of preanal papillae.

The differences between the above description and that of Baylis are not considered of specific importance, especially as the postanal papillae are exactly as he describes. The differences concern chiefly the size of the spicules, the shape of the dorsal lip and the simple, instead of double, papillae on that lip. Baylis does not mention the number of preanal papillae, but Hsü and Hoeppli (1931), reporting on *O. filaria* from *Python molurus* in China, give the number as 38–40 pairs. They also note slight differences from Baylis' description, such as the relative length of the tail and the length of the spicules. The latter character is apparently extremely variable within the species.

Three of the worms in the present material were immature and their head ends were embedded in a nodule formed from the gastric mucosa. In a note sent with the collection Purvis states, "Python ex oesophagus, stomach and small intestine. These worms occur mostly in nodules—though some of the small ones are free. The heads may penetrate through gut and peritoneum to show in the peritoneal cavity." In seven of the tubes examples of immature Ophidascarids in various stages of development were found, some of them in the act of moulting. The contents of these tubes came from different parts of the body—liver, lungs, oesophagus, stomach and intestine—and there would thus seem no doubt that the Ascarids of snakes undergo a migration in the body of their host similar to that of the Ascarids of Mammals.

O. baylisi n.sp. (Figs. 1 and 2)

This species was collected from the stomachs of at least two pythons.

Female: Up to 115 mm. in length, maximum breadth 2·5 mm. The lips are almost square with truncated corners. Each ventro-lateral lip has one large double papilla near its ventral side. The interlabia are well marked and the basal grooves deep and extensive, those at the base of the dorsal lip practically meeting in the middle line. The dorsal lip is indented in front and bears no dentigerous ridges. It has two double papillae situated about half-way along its length. The pulp has a characteristic shape, with three main horns and a number of smaller ones, branching out, antler-wise, from the chief pulp mass on each side. The oesophagus is rather short—about 3·0 mm.

The vulva is situated within the posterior third of the body, about 35 mm. from the hind end. The tail is rounded, without a terminal spike. The eggs are nearly spherical and measure 0·09 by 0·08 mm. The shell is finely sculptured.

Male: 80 mm. long, 2·0 mm. in maximum breadth. The tail ends in a small rounded knob. The spicules are equal, with blunt tips. They are 1·6 mm. long and 0·07 mm. in maximum breadth. There are three pairs of postanal papillae, one large pair immediately behind the cloaca and two pairs farther

back, situated more laterally. The preanal papillae number thirty pairs, becoming wider apart farther forward and following a somewhat zigzag line.

The present species closely resembles *O. excavata*, described by Hsü and Hoeppli (1931) from *Python molurus* in China, particularly in the basal grooves, which in both forms nearly meet one another in the middle line at the base of the dorsal lip. One of the outstanding differences is the length of the spicule, which is the smallest hitherto described in the genus. Specimens have been deposited in the collection of the Molteno Institute (No. 772) and also in the British Museum.

Genus **Polydelphis**

? P. anoura Duj. 1845

A few specimens, not quite mature, of this species were collected from the stomach and oesophagus of a python at Alor Star. This constitutes a new host record, the worm having been encountered previously only in two species of the genus *Python* (*P. molurus* and *P. sebae*).

P. attenuata Molin 1858

This species is apparently common, having been collected from pythons on four occasions.

P. bicornuta n.sp. (Fig. 3)

A small number of worms from the small intestine constitute a new species. There were four females and only one male, which had unfortunately been damaged at its hind end, so that some uncertainty exists about the exact number of preanal papillae.

Female: 50 mm. long, 2 mm. in maximum breadth. The worms have the typical *Polydelphis* shape. The lips are narrow, tapering in front, and longer than broad. They are markedly indented at their anterior margins. Marginal dentigerous ridges present, with extremely fine teeth. The pulp of the dorsal lip has a peculiar shape, with two lateral horns, one on each side, which diverge in front, the gap between them being partly occupied by a well-defined, rounded median lobe. The dorsal lip has two prominent simple papillae, placed about half-way along the length of the pulp. Each ventro-lateral lip has one large double papilla towards its ventral side. Length of oesophagus about 5·3 mm. Its posterior end is slightly enlarged.

The vulva lies at a point 16 mm.—*i.e.* about one-third the body length—from the front end. The ovarian coils do not extend quite as far forward as this point, but they reach backward to the hind end of the body. There are, as usual, four uterine branches. The hind end is rounded, without a terminal spike. The eggs are almost spherical, with diameters of 0·05 and 0·06 mm.

Male: 46 mm. long, 2 mm. in maximum breadth. The tail ends in a small spike. The spicules are equal, 5·2 mm. long, and very slender. There are four pairs of postanal papillae—one pair subventral, just behind the cloaca, the

other three more lateral and nearer the tail. There are at least twenty pairs of preanal papillae. Specimens have been deposited in the collection of the Molteno Institute (No. 771) and in the British Museum.

OXYUROIDEA

OXYURIDAE

Oxyurinae

Genus **Auchenacantha** Baylis 1929

Four species of Oxyuridae were found in large numbers in the large intestine of a flying lemur at Tanjong Pan. The females were very much more numerous than the males, of which only three kinds were discovered in the material. Thus the description of one of these species is unfortunately deficient.

The worms belong to the genus *Auchenacantha*, which was established by Baylis in 1929 to include two Oxyurids from *Galeopithecus volans variegatus* in Java (*Auchenacantha galeopteri*, type species, and *A. spinosa*). The genus also embraces two forms described earlier from different species of flying lemurs, one (*Oxyuris coronata*) by von Linstow (1903) from *Galeopithecus volans* in Siam, and the other (*Oxyuris corollata*) by Schneider (1866) from *Galeopithecus philippinensis* in the Philippines. In 1931 Chu described two species of Oxyurids from *G. volans* in the Philippines and erected for them a new genus, *Hoepplius*. His descriptions and figures leave no doubt that this genus is identical with Baylis' *Auchenacantha*, which must take precedence. Chu names his two species *Hoepplius spinosus* and *H. boholi*; and since, in the case of the first of these, the change in generic name means the employment of a feminine specific name (*spinosa*) already employed by Baylis for one of his species, it is proposed to alter the name *Hoepplius spinosus* to *Auchenacantha hoepplii*. *Hoepplius boholi* becomes *Auchenacantha boholi*.

The following species then come under the generic name, *Auchenacantha*:

A. galeopteri (Baylis 1929) (type species)	*Galeopithecus volans variegatus*
A. spinosa (Baylis 1929)	*Galeopithecus volans variegatus*
A. corollata (Schneider 1866)	*Galeopithecus philippinensis*
A. coronata (von Linstow 1903)	*Galeopithecus volans*
A. hoepplii (Chu 1931)	*Galeopithecus volans*
A. boholi (Chu 1931)	*Galeopithecus volans*
A. magna n.sp.	*Galeopithecus volans peninsulae*
A. parva n.sp.	*Galeopithecus volans peninsulae*
A. purvisi n.sp.	*Galeopithecus volans peninsulae*

The four species in the present material are: *A. galeopteri*, *A. parva*, *A. purvisi*, *A. magna*.

A. galeopteri Baylis 1929

The material offered an opportunity for the re-examination of the type species, and the results of this, together with certain characters in the three

new species, necessitate some alterations in the generic diagnosis given by Baylis. In the first place, the cuticle in *A. galeopteri* is annulated, as he describes, but does not bear spines. The specimens were very variable in the degree of contraction and wrinkling which the cuticle had undergone. In the best preserved specimens the cuticle is quite clearly devoid of spines, but in many worms it is longitudinally wrinkled, giving an appearance, along the edges of the folds, of rather distinct backwardly projecting spines. An examination of the co-type specimens deposited in the Molteno Institute (No. 628) has confirmed this. Two of the new species described below (*A. parva* and *A. purvisi*) are devoid of spines, and in his description of *A. boholi* Chu (1931) does not mention spines, but simply says "cuticula with transverse striations." The spines then are not diagnostic of the genus. A further point relating to the cuticle is that in *A. purvisi* the striations are very indistinct.

In the second place, the head end of the male is different from that of the female. Baylis' material included males of *A. galeopteri*, but he gives no specific description of their anterior ends; no males of *A. spinosa* were found. Chu's material included the males of *A. hoepplii* only, and he states that they have six lips like the females. The males in my material, including those of *A. galeopteri*, show quite clearly a three-lipped condition which contrasts strongly with that in the females.

In the third place, the flap on the end of each lip is by no means always turned outwards, but in some species lies quite flat.

The generic diagnosis then must be restated as follows:

Oxyurinae: Mouth with six lips in the female, each of which is sometimes produced into an outwardly curved cuticular flange, and either six or three lips in the male. Oesophagus with an anterior swelling containing a valvular apparatus and a pyriform posterior bulb also containing valves. A more or less distinct "pharynx" present. Lateral alae present, either in the cervical region or behind it. Cuticular annulations more or less highly developed in the cervical region, where they may be quite smooth or armed with backwardly projecting spines, arranged in a varying number of longitudinal series. When present these spines are much less prominent in the male. Posterior end of male with a cuticular expansion supported by three ray-like structures, and with a small number of papillae in the vicinity of the cloaca. In front of the cloaca a median ventral process. A single spicule present. No accessory piece. Tail of female long and tapering. Vulva sometimes prominent, generally considerably in front of the middle of the body. Eggs oval or elongate.

Description of new species

In this material, as stated above, there were four distinct kinds of females and only three kinds of males. One kind of each sex was identified as *A. galeopteri*. This leaves three females and two males. One of the females was a "spiny" *Auchenacantha*, which should have a "spiny" partner, judging by the only male so far described (Chu, 1931). Since none of the males in the

present collection had spines, it was concluded that only females of this species were present. It is impossible to be quite sure which of the remaining pairs of males and females should go together, as none of the worms were found *in copula*, in spite of careful handling. However, since there were two distinct sizes of worms of both sexes, it was assumed that the smaller males belonged to the smaller females.

Auchenacantha parva n.sp. (Figs. 6, 7, 12, 13, 16–20)

Female: Length 7·9 mm. Tail 1·96 mm. long, very slender. The vulva is situated 1·52 mm. from the anterior end and is not prominent. The cuticle is transversely striated, the annulations stand out very little from the surface and are not imbricating. In the cervical region they are about 0·019 mm. apart, but become closer together farther back. They extend almost to the tip of the tail and are interrupted laterally by the alae. There are no spines. The lateral alae begin on each side of the head and extend backwards, gradually losing themselves in the tail region. In the neck each ala is about 0·035 mm. wide. The head end is rounded and the buccal opening hexagonal. The six projections from its edge are not usually scroll-like and are rather short. There are no "lappets" between them (see *A. magna*). There are two cephalic papillae on the sides of the head, facing antero-laterally. The pharynx and oesophagus have the typical *Auchenacantha* structure. The oesophagus is 1·0 mm. long, *i.e.* its posterior bulb is about 0·52 mm. in front of the vulva. The eggs are oval, symmetrical and slightly flattened at one pole. They measure 0·04 by 0·023 mm.

Male: 2·30 mm. long and normally curved ventrally into a bow. General structure typical of the genus, but the ventral process is very markedly bent backwards (Fig. 18; compare *A. galeopteri*, Fig. 26). The single spicule is poorly chitinised. It is 0·03 by 0·005 mm. The oesophagus is approximately one-quarter the body length. Lateral alae as in the female. The head bears two lateral cephalic papillae, as in the female, but there are only three lips, which are very short and not turned outwards. The pharynx is three-sided and its walls are strongly chitinised.

Auchenacantha purvisi n.sp. (Figs. 8–11, 21–25)

Female: Length 9·0 mm. Tail 2·0 mm. long. Vulva prominent and situated 1·24 mm. from the anterior end. The striations on the cuticle are excessively fine and very indistinct. There are no spines. The lateral alae have the same extent and dimensions as in the preceding species. The anterior end is flat in front and the whole head is surrounded by a turban-like expansion 1·95 mm. in diameter. This is not vesicular in structure, but is filled with pulp tissue. The mouth is circular and the six lips do not end in scroll-like structures, but run directly inwards towards the mid-line, narrowing the mouth opening to a six-rayed star. There are no intermediate "lappets." Owing to the flatness of the front of the head the two lateral cephalic papillae face directly forwards.

The pharynx has the same structure, except for unimportant details, as in *A. parva*. The oesophagus is 1·3 mm. long and thus its posterior bulb is at about the same level as, or even slightly behind, the vulva. Eggs oval, symmetrical, 0·046 by 0·028 mm.

Male: The putative male of this species does not possess the expanded head end characteristic of the female, but it differs from the other males in being normally rolled into a more or less tight spiral, except at the anterior end, which is straight. Apart from this it has the typical *Auchenacantha* structure, the chief distinctive points being as follows: it measures 3·80 mm. in length and the spicule is 0·08 by 0·009 mm. The ventral process is more sessile than in the other males, not pedunculated, but broader at the base than at the tip.

Auchenacantha magna n.sp. (Figs. 4, 5, 14, 15)

Female: Length 13·4 mm. Vulva 2·3 mm. from the anterior end, prominent. The average length of the tail is 7·4 mm., but this is extraordinarily variable. Cuticular striations very well marked, very broad in the head and cervical region and slightly imbricating. In the anterior part of the body they bear large spines. The first ring behind the mouth has no spines, the next twelve. Proceeding backwards, the spines increase in number (up to about twenty-three) and decrease in size, becoming farther apart. They are backwardly hooked. They cease abruptly at a point about 2·0 mm. from the head end, and this point is followed by about ten very closely set transverse striations. Immediately behind these the lateral alae begin. They run backward to about half-way along the tail. The anterior end of the body is practically a flat surface, in the centre of which the mouth opens. It is surrounded by six scroll-like lips and between them are six smaller pointed "lappets." These have not so far been described in other species. There is a single large cephalic papilla on each side of the head, facing directly forward. The pharynx appears to be shorter and less well developed than in the other species. The oesophagus is 1·2 mm. long—*i.e.* relatively short, so that the posterior bulb is well in front of the vulva. Eggs asymmetrical, flattened on one side and very elongated. They measure 0·07 by 0·022 mm.

Male: Unknown.

Specimens of the three new species of *Auchenacantha* described in this paper have been deposited in the collection of the Molteno Institute (Nos. 769, 770 and 768) and in the British Museum, South Kensington.

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EXPLANATION OF PLATES XXIV–XXVI

PLATE XXIV

Fig. 1. *Ophidascaris baylisi*, ♀; dorsal view of head.
Fig. 2. *O. baylisi*, ♂; tail, side view.
Fig. 3. *Polydelphis bicornuta*, ♀; dorsal view of head.
Fig. 4. *Auchenacantha magna*, ♀; complete.
Fig. 5. *A. magna*; egg.
Fig. 6. *A. parva*, ♀; complete.
Fig. 7. *A. parva*; egg.
Fig. 8. *A. purvisi*, ♀; complete.
Fig. 9. *A. purvisi*; egg.

Scale (*a*) refers to Figs. 4, 6 and 8; scale (*b*) to Figs. 1 and 2; scale (*c*) to Fig. 3.

PLATE XXV

Fig. 10. *A. purvisi*, ♀; dorsal view of head.
Fig. 11. *A. purvisi*, ♀; front view of head.
Fig. 12. *A. parva*, ♀; dorsal view of head.
Fig. 13. *A. parva*, ♀; front view of head.
Fig. 14. *A. magna*, ♀; dorsal view of head.
Fig. 15. *A. magna*, ♀; front view of head.

PLATE XXVI

Fig. 16. *A. parva*, ♂; front view of head.
Fig. 17. *A. parva*, ♂; complete.
Fig. 18. *A. parva*, ♂; ventral process.
Fig. 19. *A. parva*, ♂; ventral view of bursa.
Fig. 20. *A. parva*; lateral view of spicule.
Fig. 21. *A. purvisi*, ♂; front view of head.
Fig. 22. *A. purvisi*, ♂; complete.
Fig. 23. *A. purvisi*, ♂; ventral process.
Fig. 24. *A. purvisi*; lateral view of spicule.
Fig. 25. *A. purvisi*, ♂; ventral view of bursa.
Fig. 26. *A. galeopteri*, ♂; ventral process.

Scale (*a*) refers to Figs. 18, 19, 23, 24, 25 and 26; scale (*b*) to Figs. 17 and 22.

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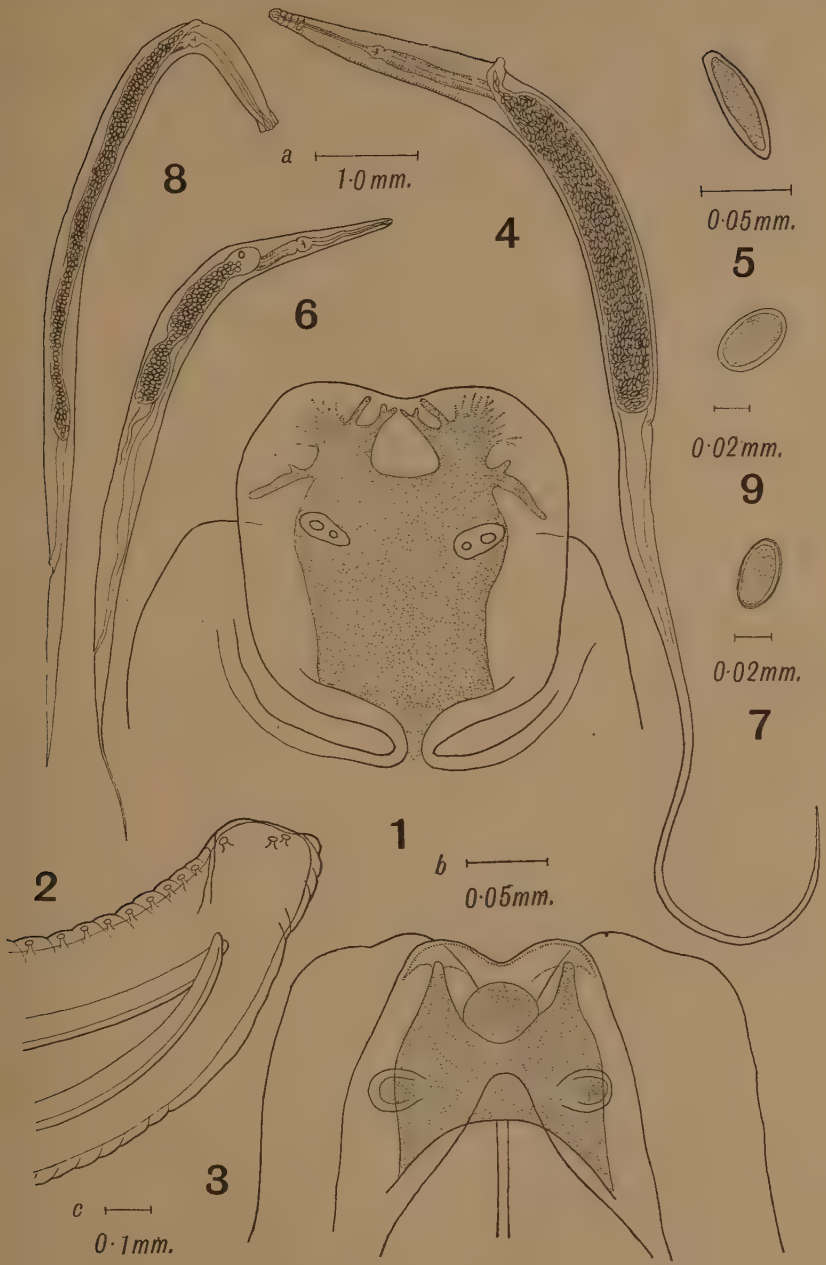
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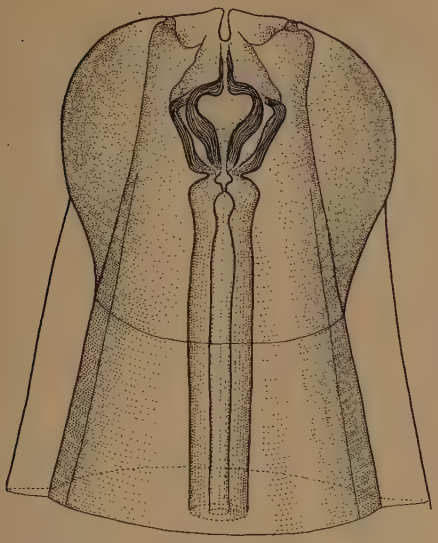

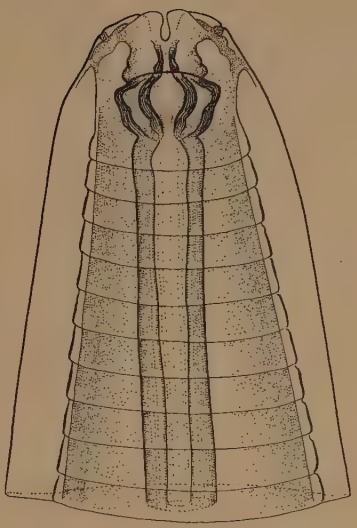

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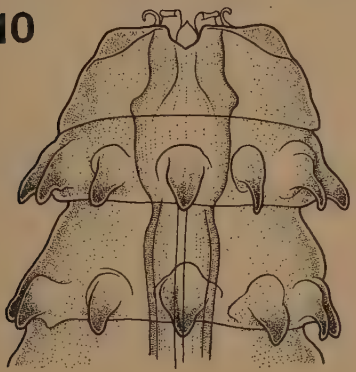

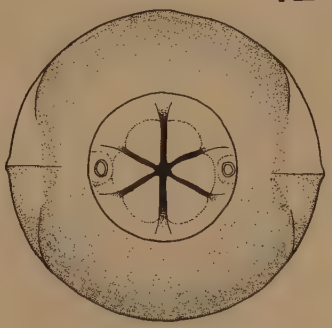

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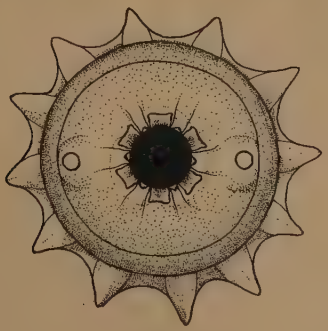

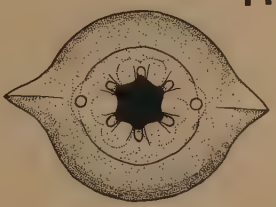

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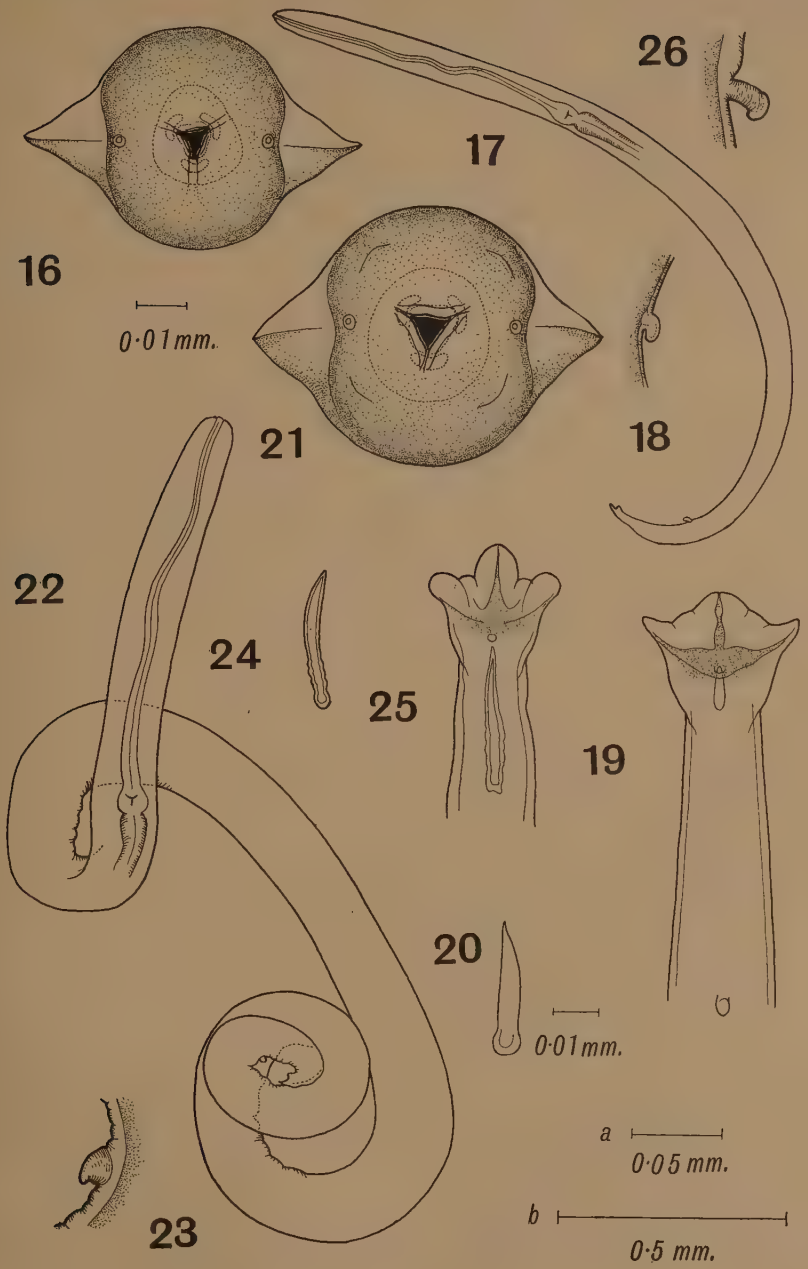
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ECOLOGICAL STUDIES ON SOME TICKS

By F. S. BODENHEIMER

Head of the Department of Zoology, Hebrew University, Jerusalem

(With 3 Figures in the Text)

Many basic data relating to the ecology of ticks are known, thanks mainly to the studies of Hunter, Bishopp and others on North American ticks.

The larvae and adult ticks are well known to be extremely resistant to influences of climate and hunger. It is therefore probable that their most sensitive stage, which is mostly influenced by environmental factors, is the egg or the early larval stage before feeding. The aim of this paper is to contribute towards the knowledge pertaining to the tolerance the ticks show towards the climate while in these stages. The question of how far climate and food conditions influence egg maturation and oviposition, processes which possibly go to make a second sensitive stage, was not studied here.

The breedings were performed in the slightly changed multiple thermostat of Williams. Air humidity was controlled by sulphuric acid in different concentrations. Two or three series of 80–120 eggs each were exposed to the different combinations of temperature and humidity. The given values are the average of all series.

(1) **Ornithodorus coniceps** Can.

This fowl tick is restricted in Palestine to the hill country. The shortest duration of development of its eggs in days is:

Temp. °C.	Relative humidity (%)				Average shortest development	Average development
	70	80	90	100		
10	0	0	0	0	0	0
15	0	0	0	47	47	48
18	0	0	0	39	39	39·6
20	0	29	24	22	25	27·0
22	0	0	17	18	17·5	—
24	0	17	18	15	17	18·7
30	0	13	13	11	12·3	—
33	0	0	12	11	11·5	—
35	0	0	12	10	10·3	—
37	0	0	7	0	7	11·6

Some breedings, which developed very quickly at higher temperatures, are not included in these homogeneous results because of their strong divergence. They are:

Temp. °C.	Relative humidity (%)			Average
	80	90	100	
28	6	10	7	7·7
30	5	4	4	4·3
33	—	7	7	7·0

The development threshold of the normal series is at 10·2° C. for the shortest, at 10·6° C. for the average development. The thermal constants are respectively 226 and 211 day-degrees. A relative humidity of 100 per cent. is optimal and drier air lengthens the development.

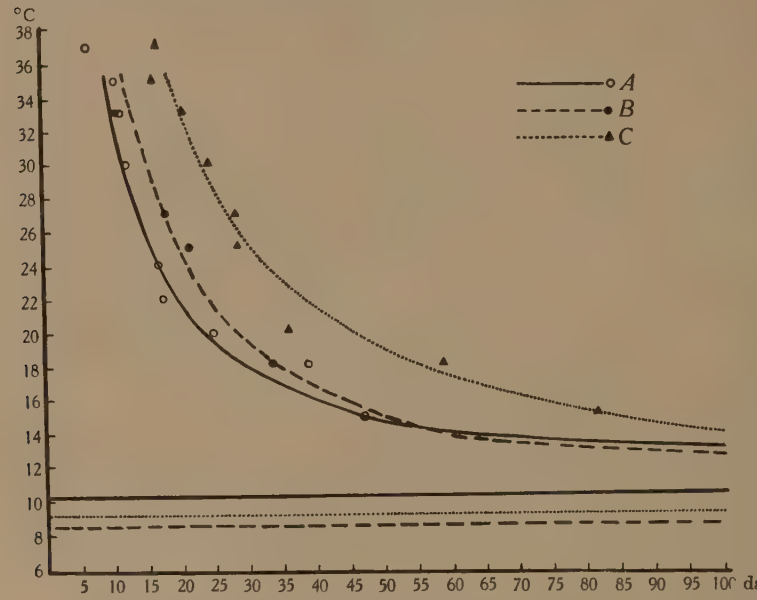

Fig. 1. The hyperbolic curve, representing the temperature dependency of development of eggs of *Ornithodorus coniceps* (full line), *Argas persicus* (interrupted line) and *Hyalomma aegyptium* (in dotted line). The points are the empirical values.

The shortest development is at 35° C. Above 37° C. no complete development takes place in the egg.

The number of larvae hatched per 100 eggs at the different combinations of temperature and humidity was:

Temp. ° C.	Relative humidity (%) 60	70	80	90	100	Total
7	0	0	0	0	0	0
10	0	0	0	0	0	0
15	0	0	0	0	5·0	5
18	0	0	0	0	7·5	7·5
20	0	0	2·5	10	47·5	60
24	0	0	25	10	32·5	67·5
28	0	0	40	62·5	*77·5*	*180*
30	0	0	27·5	40	65	132·5
33	0	0	0	10	12·5	22·5
35	0	0	0	2·5	25	27·5
Totals	0	0	95	135	*272·5*	

The totals given in this table are insignificant in themselves, but they clearly illustrate again that the eggs show much more tolerance towards tem-

perature changes than towards those of humidity. Eighty per cent. relative humidity is the minimum, 100 per cent. the optimum condition. The vital optimum is at 28° C. and 100 per cent. relative humidity.

The mean longevity of freshly hatched larvae (two series of twenty larvae each) in days was:

Temp. ° C.	Relative humidity (%) 60	70	80	90	100	Total
7	0	0	0	0	0	0
10	0	0	0	0	0	0
15	0	0	0	0	30·5	30·5
18	0	0	0	0	9·7	9·7
20	0	0	13·1	13·4	18·5	45·0
24	0	0	12·6	16·2	*21·3*	*50·1*
28	0	0	15·6	12·9	20·3	48·8
30	0	0	11·5	11·8	14·3	37·6
33	0	0	0	7·1	8·4	15·5
35	0	0	0	5·5	8·2	13·7
Totals	0	0	52·8	66·9	*131·2*	

These larvae have been hatched from eggs at the vital optimum and have never been fed. The long life duration at 15° C. is not conditioned by optimal conditions, but by the retardation of life processes at low temperatures. The vital optimum of the freshly hatched, unfed larvae, which stage is very important for the ecology of the species, is at 24° C. and 100 per cent. relative humidity, *i.e.* 4° C. lower than that for the development of the eggs.

The scale of experimental activity is:

Stage	No. of observations	1* ° C.	2 ° C.	3 ° C.	4 ° C.	5 ° C.	6 ° C.	7 ° C.	8 ° C.
Large nymphs	21	6·1	15·8	15–21	21·5	28·0	40·1	40·5	44·8
Adults	9	10·7	14·3	16·5–19·5	20·3	33·0	37·1	43·5	45·5

* The scale expresses: (1) beginning of cold torpor, (2) only weak movements, (3) only interrupted crawling, (4) beginning of normal activity, (5) beginning of high activity, (6) beginning of irritative activity, (7) beginning of heat paralysis, (8) instantaneous heat death.

The preference temperature of the nymphs is at 25·7° C., for the adults at 26·5° C. ($n = 212$; Fig. 2)[1].

(2) **Argas persicus** Fisch.

This dangerous fowl tick occurs mainly in the lowlands. The development threshold for its egg stage is at 8·5° C., the thermal constant at 316 day-degrees. No decisive influence of the relative humidity is recognisable. The average shortest development of the different series of egg breeding (in days) was:

Temp. ° C.	Relative humidity (%) 20	40	60	70	80	90	100	Average
18	31·3	34·7	36·8	34·5	34·5	35·0	34·1	33·3
25	24·0	23·3	23·4	21·0	22·4	21·6	20·0	22·2
27	16·0	0	16·0	16·0	18·0	18·0	18·0	17·0
33	0	0	10·0	9·5	0	0	12·0	10·5

[1] The apparatus and procedure is described in *Zeitschr. vergl. Physiol.* **8**, 1–15, 1928.

The number of larvae which hatched successfully from 100 eggs at different combinations of temperature and humidity was:

Temp. °C.	Relative humidity (%)						
	20	40	60	70	80	90	100
10	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0
18	70	70	75	75	85	80	60
20	0	0	0	0	*95*	90	85
25	65	85	50	50	80	75	85
27	82	0	75	90	85	75	75
33	0	0	15	60	0	0	56

The vital optimum of the egg stage of *Argas persicus* is 20° C. and 80 per cent. relative humidity. Their very large tolerance of climatic factors is im-

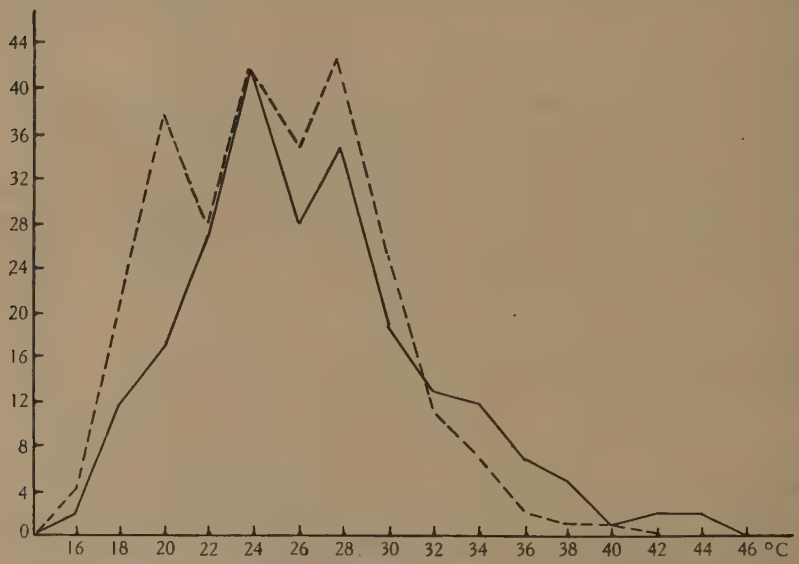

Fig. 2. The preference temperature of *Ornithodorus coniceps* (full line, adults; interrupted line, nymphs), representing the number of individual readings for every temperature in a temperature gradient.

portant for the understanding of the large distribution of this eurobiotic species. Even at 20 per cent. relative humidity the mortality is only very little higher than in the vital optimum.

The scale of activity of the adults is:

No. of observations	1 °C.	2 °C.	3 °C.	4 °C.	5 °C.	6 °C.	7 °C.	8 °C.
59	2·6	6·9	12·0	20·4–25·1	33·7	40·2	46·4	47·7

The experiments in a temperature gradient were unsuccessful, as neither nymphs nor adults responded to the changing stimuli of temperature.

(3) **Hyalomma aegyptium** L.

This tick also is largely distributed. Its larvae approach the traveller in the desert, wherever he camps or reposes on camel tracks. A high tolerance towards environmental influences would be expected *a priori*. The average shortest length of development of its eggs (in days) was:

Temp. °C.	Relative humidity (%)							
	20	40	60	70	80	90	100	Average
15	0	0	78	80	76	65	76	75·0
18	0	0	50	53	53	54	55	53·0
20	0	0	34	33	33	35	35	34·0
25	0	0	27	27	28	28	28	27·6
27	0	28	0	27	0	27	28	27·5
30	0	22	22	22	23	23	24	22·8
33	0	20	0	20	20	20	0	20·0
35	0	0	15	0	15	15	20	16·3
37	0	0	0	0	0	0	16·5	16·5
40	0	0	0	0	0	0	0	0

The threshold of development is at 8·9° C., the thermal constant 485 day-degrees. The influence of atmospheric humidity upon its duration is negligible. The tolerance of the egg stage of this eurobiotic species is very high. From 100 eggs hatched successfully at a given relative humidity and temperature:

Temp. °C.	Relative humidity (%)							
	20	40	60	70	80	90	100	Total
10	0	0	0	0	0	0	0	0
15	0	0	7·5	38·9	35·0	37·5	40·0	158·9
18	—	—	22·5	42·5	82·5	61·3	77·5	286·3
20	1·3	2·5	10·0	30·0	67·5	62·5	80·0	253·8
25	0	3·8	70·0	80·0	77·5	75·0	75·0	381·3
27	0	12·5	0	76·3	65·0	93·8	91·3	338·9
30	0	22·5	62·5	75·0	95·0	*100·0*	95·0	450·0
33	0	25·0	0	47·5	82·5	85·0	60·0	300·0
35	0	0	72·5	0	92·5	95·0	32·5	292·5
37	0	0	0	0	0	0	47·5	47·5
40	0	0	0	0	0	0	0	0
Totals	1·3	66·3	245·0	390·2	597·5	610·1	598·8	

The tolerance towards climatic influences is large, but decidedly smaller than in *Argas persicus*, especially at humidities below 40 per cent. The vital optimum is at 30° C. and at 90 per cent. relative humidity. The scale of activity is for:

Stage	No. of observations	1 °C.	2 °C.	3 °C.	4 °C.	5 °C.	6 °C.	7 °C.	8 °C.
Larvae (2–3 days old)	20	6·4	8·1	12·0	18·0–22·5	33·7	44·6	51·5	51·7
Adult	30	0·3	8·3	14·2	19·8–24·3	31·8	38·5	46·0	48·3

No preference temperature could be recorded owing to the immobility of the species in the temperature gradient.

DISCUSSION OF THE RESULTS

The climatic limitations of *Ornithodorus coniceps* are obvious. Egg and freshly hatched larva are both in need of a high air humidity. The species is

known from the Mediterranean region to Cape Colony. The species can resist fairly low winter temperatures, but its development threshold is somewhat higher than that of the other species. Vital optimum and preference temperature do not agree very well with the Mediterranean macroclimate, but agree fairly with the microclimate of their environment in Palestine, which has been described by Theodor. The low thermal death-point coincides with the nocturnal habits of the species.

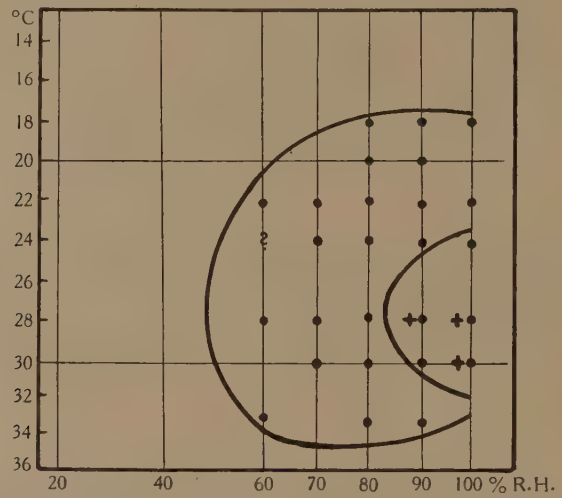

Fig. 3. The temperature-humidity range, in which 50 per cent. and more of the eggs develop successfully, + *Ornithodorus coniceps*; ● *Hyalomma aegyptium*, is represented by the two curves. The values for *Argas persicus* have not been entered in the design, as it would embrace practically the whole range between 20 and 100 per cent. relative humidity and 18–33° C.

Argas persicus, which is widely distributed in both subtropical and tropical countries, is very resistant to climatic influences. The climatic mortality neither of the egg nor of the early larval stage can be regarded as a limiting factor in this species. Despite a development threshold of 8·5° C. no egg finished its usual development. The vital optimum is at 20° C. at 80 per cent. relative humidity. The shift in the optimum of humidity is important, but the lowering of its temperature is probably only based on the small number of experiments and should be at about 27° C. The influence of the combined climatic factors is lower in this species than in any other animal studied before. The low mortality even at 20 per cent. relative humidity is in good agreement with life history and conditions. For this, as well as in the following species, the maximal range of activity is much larger (45° C.) than in the preceding species (37° C.). This coincides well with the large daily temperature range in steppes and deserts.

The values and the distribution for *Hyalomma aegyptium* are similar to those of *Argas persicus*. The higher thermal death-point is explained by the

diurnal habits of *Hyalomma*. This tick, which is fairly common even in deserts, is very tolerant towards temperature, but less tolerant towards low humidity than *Argas*; but at 20 per cent. relative humidity 1·3 per cent. of the eggs still developed in one case, and the large number of more than 10,000 eggs permits the maintenance of the species even under very unfavourable conditions. Besides climatic factors the accessibility of the host is of primary importance for the fluctuations of tick populations. Many observations seem to show that the rôle of larvae and nymphs in search of the host is by no means very passive. Even in the desert resting mammals soon form an invasion centre for the stages of the ticks for an area the size of which is still to be determined. Quantitative experiments in this respect are highly desirable.

The most important biological constants

	Ornithodorus coniceps ° C.	*Argas persicus* ° C.	*Hyalomma aegyptium* ° C.
Development threshold of the egg at	10·2	8·5	8·9
Shortest development of the egg at	30·0	—	*ca.* 35·0
Vital optimum (egg)	28 (100 % R.H.)	20 (80 % R.H.)	30 (90 % R.H.)
Vital optimum (freshly hatched larvae)	24 (100 % R.H.)	—	—
Instantaneous heat death	45·2	47·7	51·7
Beginning of cold torpor	8·4	2·6	6·4
Preference temperature	25·9	—	—
Extreme range of activity (Cold torpor/Heat paralysis)	36·8	45·1	45·3
Normal range of activity (Beginning of normal to that of irritative activity)	17·0	19·7	26·6

The following results have been arrived at with regard to the life history of the three species in Palestine:

In *Ornithodorus coniceps* development is very slow during winter and is interrupted for some time. This species can develop its eggs only in the humid spring. At all other seasons, with the only exception of very special microclimates, 100 per cent. of the eggs would die. The other species can develop successfully in all stages from spring to autumn and they actually do so.

The writer wishes to express his thanks to Dr O. Theodor for help he extended in getting the ticks and for his advice on their breeding technique.

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NAIROBI SHEEP DISEASE: NATURAL AND EXPERIMENTAL TRANSMISSION BY TICKS OTHER THAN *RHIPICEPHALUS APPENDICULATUS*

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(With 3 Charts in the Text)

Montgomery (1917) showed that Nairobi sheep disease was commonly transmitted by *Rhipicephalus appendiculatus*. In 1931 we confirmed Montgomery's findings and extended them considerably in respect of transmission by this species of tick. It was shown that infection of any instar would result in transmission by the next succeeding stage, which would then in the normal course lose its infection during the next moult. A female tick infected in this manner as an adult would pass infection through the eggs to the larvae of the next generation. Further it was demonstrated that the transmitting stage could be reinfected if the reaction of the host animal commenced before the infecting meal was completed, thus ensuring the carrying on of infection for a further stage.

Referring to possible transmission by ticks of other species, Montgomery expressed the opinion that further work was required to negative this possibility. Apparently his tests of other species included only the feeding of two adult *Amblyomma variegatum* that had been collected as nymphae from naturally infected animals. These two ticks did not transmit Nairobi sheep disease when fed on a susceptible animal, although adult *Rhipicephalus appendiculatus*, collected as nymphae from the same animals during the same period, transmitted the disease to at least one susceptible sheep.

During the year 1931 the possibility that Nairobi sheep disease might be transmitted by ticks other than *R. appendiculatus* was brought very forcibly to our notice by the occurrence of several outbreaks of the disease in sheep that were not infested with this species. The history of these outbreaks is as follows: In March, 1931, an extensive outbreak of Nairobi sheep disease occurred on and around the Laikipia plains to the west of Mt Kenya. Losses on one large Merino sheep farm were at the rate of six hundred a month over a period of eight weeks. On another farm the owner stated that between three and four hundred sheep died of the disease during a month. Sheep from these and from other adjoining farms were brought to the laboratory for autopsy. Lesions were found which resembled those of Nairobi sheep disease in their situation and in their general nature, but there was a noticeable difference in the severity of these lesions. The spleen *tumor*, for example, was

not so marked, nor were the petechiae in the left heart and abomasum as extensive as in typical cases of Nairobi sheep disease. Lesions in the kidney were restricted to a slight radial congestion of the cortex and boundary layer; but the lesions in the mucosa of the large intestine were fairly typical. Many of the sheep had numerous *Oesophagostomum* nodules in the wall of the caecum and colon; and it was remarked that with the development of lesions of Nairobi sheep disease there was a strong tendency for the haemorrhages to be particularly intense in the neighbourhood of the old nodules, and for the quiescent nodules themselves to appear active and strongly congested.

The nature of the infection was definitely established by the inoculation of susceptible sheep, and by subsequent cross-immunity tests with the laboratory strain of virus. Virus from three different outbreaks was tested in this manner (*vide* Table I).

Table I. *Cross-immunity experiments between Laikipia and Athi Plains viruses and laboratory Nairobi sheep disease virus.*

Source of virus	No. of sheep inoculated and history	Result of inoculation	Result of immunity test with laboratory strain
Laikipia	Naturally contracted case	—	Immune
,,	3315—susceptible	Reacted	,,
,,	3528— ,,	,,	,,
,,	3143—immune	No reaction	—
,,	3530— ,,	,,	—
Sheep 3315	3067—susceptible	Reacted	Killed for autopsy
,, 3315	3602— ,,	,,	Immune
,, 3315	3608— ,,	,,	,,
,, 3315	3284—immune	No reaction	—
—	3816—control for immunity test	—	Reacted
—	3832— ,,	—	,,
Athi Plains	3951—susceptible	Reacted	Immune
,,	3983— ,,	,,	,,
,,	3959—immune	No reaction	—
,,	3991— ,,	,,	—

The three farms in question are situated within what is known as the northern clean area, that is to say clean in respect of East Coast fever. We were familiar with the tick fauna of this region and well aware that *R. appendiculatus* was not normally present. During the outbreaks several collections of ticks were examined from infected flocks and *R. appendiculatus* was never found. On sheep, the most numerous tick over the whole area was *R. evertsi*. On one estate bont-legged ticks, *Hyalomma aegyptium* and *H. aegyptium impressum*, were next in order of frequency; but in other parts of the affected area *Hyalomma* spp. were absent at that time. On all the farms *Rhipicephalus simus* was the next most frequent species, and *R. pulchellus* was fairly numerous in certain parts of the area. *Amblyomma variegatum* was widely distributed but never numerous.

In August and November of the same year two other outbreaks of Nairobi sheep disease were encountered on farms that were not infected with *Rhipicephalus appendiculatus*. These farms are situated on the Athi Plains to the south-east of Nairobi. The adult ticks present on sheep in this locality were,

in order of frequency, *R. simus*, *R. pulchellus*, *Amblyomma variegatum*, *Rhipicephalus evertsi* and *R. bursa*. Of nymphae collected from sheep and goats on the first of these farms the great majority proved after moulting to be *R. evertsi*. A few nymphae of *R. pulchellus* and *R. bursa* were also collected.

In spite of our failure to demonstrate the known vector of the virus in either of the affected localities owners were advised to resort to the usual preventive measures, thorough hand-dressing with tobacco extract suspended in crude oil coupled with movement to fresh pastures where possible. This procedure proved completely successful in controlling the spread of the disease.

It was then decided to submit each tick species collected from sheep during these outbreaks to tests for infectivity in respect to the virus.

R. simus, abundantly present in both localities and most closely related to *R. appendiculatus*, was naturally the most suspected tick and consequently the species first tested.

Rhipicephalus simus Koch 1844

According to Lounsbury (1906) (in Lewis, 1932) the ox is not a suitable host for *simus* larvae, although the nymphae and adults do not dislike cattle. Lewis (1932) states that the hare does not appear to be the usual host for the larval stage and that larvae do not readily attach themselves to the ears. Of larvae about 40 per cent. only fed to repletion on hare. Lewis, however, refers to the finding of adult *simus* in the nests of the field rat, *Arvicanthis abyssinicus nairobiae*, and in smaller numbers in the nests of *Mastomys coucha panya*, *Otomys angoniensis elassodon* and *Lemniscomys striatus*. He also quotes Dr J. I. Roberts, entomologist at the Medical Research Laboratory, Nairobi, for the statement that nymphae and probably to a less extent larvae feed readily on field rats, although the adults are rarely collected on the rats themselves.

During our investigations into the susceptibility of wild rodents to the virus of Rift Valley fever, Dr Roberts kindly supplied us with comparatively large numbers of field rats trapped on the Athi Plains. It was when examining these rodents for ectoparasites that the predilection of immature *Rhipicephalus simus* to feed on field rats was first noticed. Table II shows the incidence of tick species on eighty-eight *Arvicanthis abyssinicus* and *Mastomys coucha* trapped in this area; a few of the former species trapped in the Laikipia district also furnished larvae and nymphae of *Rhipicephalus simus*.

Table II. *Infestation of wild rats with ticks.*

Species of rat	No. examined	No. infested with ticks	Total no. infested with *R. simus*	*R. simus*			No. infested with *H. leachi*	No. infested with other ticks
				Adults	Larvae	Nymphae		
Arvicanthis abyssinicus nairobiae	57	34	34	1	11	28	1	0
Mastomys coucha panya	31	3	2	—	—	2	1	0

When the Athi Plains outbreak of Nairobi sheep disease occurred, many nests of field rats were dug up and searched for ectoparasites. Table III was compiled from specimens and data supplied by Dr Roberts after an examination of 137 nests from this area. It will be observed that *R. simus* is the most frequent inhabitant of these nests, and that *Arvicanthis abyssinicus* is the most favoured host.

Table III. *Infestation of rat nests with ticks.*

Species of rat	No. of nests examined	No. of nests with ticks	No. of nests with more than one species	No. of nests with *R. simus* adults	No. of nests with *H. leachi* adults	No. of nests with *R. pulchellus* adults	Double infestations *R. simus* and *H. leachi*	Double infestations *R. simus* and *R. pulchellus*
Arvicanthis abyssinicus nairobiae	61	52	18	40	30	—	18	—
Mastomys coucha panya	44	18	5	12	10	1	4	1
Otomys angoniensis elassodon	25	14	4	7	10	1	4	—
Lemniscomys striatus	7	2	2	2	2	—	2	—

In five separate attempts we failed to induce *simus* larvae to feed on the ears of sheep. Four attempts to feed nymphae on sheep were equally unsuccessful. Partially fed adults collected from sheep suffering from naturally contracted Nairobi sheep disease re-attached themselves when placed on the ears of fresh sheep and engorged without provoking any reaction. Table IV

Table IV. *Attempts to transmit to sheep with* R. simus.

Stage	History	Sheep No.	Date placed on ear	Result
Larvae	Bred from a female collected 6. v. 31 engorged on a natural case from Laikipia. Oviposition 17. v. 31. Hatching 27. vi. 31	3290	27. vii. 31	Larvae would not feed and died. Sheep later found to have been immune
		3338	10. vii. 31	Larvae would not feed and died. Sheep remained susceptible
		3811	18. vii. 31	Larvae remained alive for seven days but would not feed and died. Sheep remained susceptible
,,	Bred from female from a natural case of the disease	3161	27. vii. 31	Several hundred placed on ear but none fed
,,	Bred from females collected partly fed on a natural case and which finished feeding on Sheep 3858 (see below)	3437	18. i. 32	Several hundred placed on ear but none fed
Nymphae	Laboratory reared	3681	5. viii. 31	Nymphae died
,,	,,	3813	10. viii. 31	,,
,,	,,	3866	,,	,,
,,	,,	3437	,,	,,
Adults	Bred from nymphae collected on *Arvicanthis* from the Athi Plains centre of disease	3960	18. xii. 31	Adults fed well. Sheep did not react and later was shown to be susceptible
,,	Collected partly fed on an infected sheep from the Athi Plains	3858	28. ix. 31	Continued to feed on new host and females eventually laid. Larvae from these females were placed on Sheep 3437 (above)

illustrates attempts to feed larvae and nymphae on sheep. It will be noticed that the larvae used in three of the experiments were bred from females collected from a natural case of the disease; they should have been infected. No difficulty was experienced in feeding *R. simus* adults on the ears of sheep.

It was evident therefore that before *R. simus* could be considered a potential vector of Nairobi sheep disease it would be necessary to prove that the virus could persist in the blood of a small rodent such as *Arvicanthis* for sufficiently long periods and in sufficient concentration to ensure infection of larval and nymphal ticks. With the object of gaining information on these points a number of *Arvicanthis*, white rats, and rabbits were inoculated with virulent blood from reacting sheep. These animals were bled or destroyed at varying periods after infection and their blood tested by the inoculation of susceptible sheep.

Two white rats were inoculated intraperitoneally with virulent blood and their blood, pooled, was intravenously inoculated into Sheep 3936 daily from the second to the fifteenth day after inoculation. The sheep did not react and was later proved to have been susceptible. Two rabbits were also inoculated intraperitoneally with virus, and were bled from the second to the twelfth day for the subcutaneous inoculation of Sheep 3841, which did not react and was later shown to be susceptible.

Table V illustrates the results obtained with *Arvicanthis* trapped in a part of the Rift Valley where Nairobi sheep disease is uncommon. It will be observed that the virus was present in the blood up to four days after inoculation. Six and thirteen days after inoculation the blood of *Arvicanthis* was not infective. A further series of experiments was undertaken to determine whether the presence of virus in the blood of *Arvicanthis* during the first four days after inoculation constituted a genuine infection, or whether the virus recovered was merely part of the inoculated virus that had not yet been eliminated from the circulation. Eight *Arvicanthis* were inoculated with a Laikipia strain of virus on February 29th, 1932, and four of them were killed three days later for separate inoculation of their blood into sheep. Table V shows that the blood of two of these four *Arvicanthis* was infective on March 3rd, 1932. The remaining four rats were reinoculated with virus eighteen days after the original inoculation and were destroyed for examination three days later. Table V shows that virus could not be demonstrated in the blood of any of these reinoculated *Arvicanthis*, and the inference is that the process is a genuine infection which confers an immunity.

Table V. *Inoculations of* Arvicanthis *with virus.*

Arvicanthis No.	Date inoculated	Date reinoculated	Date killed	Sheep inoculated	Result	Immunity test
1	18. i. 32	—	20. i. 32	3991	Reacted	Immune
2	,,	—	,,	3995	Reacted and died	—
3	,,	—	,,	3989	,,	—
4	,,	—	22. i. 32	3987	Reacted	Immune
5	,,	—	,,	3996	Reacted and died	—
6	,,	—	24. i. 32	3998	No reaction	Susceptible
7	,,	—	,,	3997	,,	,,
8	20. ix. 31	—	3. x. 31	3934	,,	,,
9	29. ii. 32	—	4. iii. 32	4010	Reacted	Not tested
10	,,	—	,,	4037	No reaction	Susceptible
11	,,	—	,,	4040	,,	Immune
12	,,	—	,,	4042	Reacted	Not tested
13	,,	18. iii. 32	22. iii. 32	4072	No reaction	Susceptible
14	,,	,,	,,	4073	,,	,,
15	,,	,,	,,	4074	,,	,,
16	,,	,,	,,	4075	,,	,,

Larvae and nymphae of *Rhipicephalus simus* were next fed on *Arvicanthis*. As was expected both stages fed well and uniformly on this host, the ticks going down immediately and completing their feed in from 90 to 120 hours. Four lots of larvae, bred from females that had engorged and dropped from Sheep 3622 during a Nairobi sheep disease reaction were fed on *Arvicanthis* 20, 54, 55 and 57. These larvae should have proved infective were *Rhipicephalus simus* capable of transmitting the virus. It will be recalled that in the case of *R. appendiculatus*, infecting ticks still contain virus when they drop off engorged after the transmitting meal irrespective of whether the host animal is susceptible or immune. It was accordingly considered worth while to inoculate sheep with these engorged larvae after they dropped from the *Arvicanthis*. Two hundred larvae were ground up and inoculated into Sheep 3984 and 3985 on the day that they dropped from *Arvicanthis* 54. Neither sheep reacted and both were proved susceptible when tested with virus. A similar number of engorged larvae from *Arvicanthis* 55 were inoculated into Sheep 3992, also on the day of dropping. This sheep did not react and was later proved susceptible. The blood of *Arvicanthis* 54 was inoculated to Sheep 3988 two days after the ticks had completed their feed. Sheep 3988 did not react and later proved susceptible. On the day that the larvae dropped the blood of *Arvicanthis* 55 also was inoculated to Sheep 3993 and 3994. Neither of these sheep reacted. Sheep 3993 proved susceptible, but 3994 gave a dubious reaction when tested.

Uninfected larvae were fed on *Arvicanthis* 22 and 23, and on the day following the infestation the rats were infected by the inoculation of virulent blood from a reacting sheep. Both rats were destroyed four days after infection, immediately after the last ticks had dropped, and their pooled blood was inoculated into Sheep 3949, 3959 and 3966. Sheep 3949 reacted and died of Nairobi sheep disease. Sheep 3959 reacted, recovered and proved immune to a subsequent test. Sheep 3966 did not react but later proved to be immune. The engorged larvae were not tested for infectivity by inoculation, since the inoculation of the blood of *Arvicanthis* 22 and 23 was sufficient proof of the infectivity of the meal.

Nymphae moulted from these larvae were ground up in saline and thirty-five were inoculated into Sheep 4087. This sheep did not react and was later proved to be susceptible. Forty-five of these nymphae were fed on *Arvicanthis* 84, which did not become infected.

Sixty-four of the remaining nymphae from these rats were fed, thirty-two on each, on two *Arvicanthis*, Nos. 82 and 83. Twenty-four hours later sixteen clean unfed nymphae, bred on *Arvicanthis* 20 and 57, were placed on *Arvicanthis* 83, and twenty-five nymphae of the same lot on *Arvicanthis* 82, the intention being that if the infective nymphae transmitted the virus to the *Arvicanthis* the second clean lot of nymphae would acquire infection from the blood. Since the ticks engorged so promptly it would be possible to collect the second batch of nymphae separately with a fair degree of certainty. The last fourteen engorged nymphae to drop were assumed to belong to the clean lot and were inoculated to Sheep 4076. This sheep did not react and was later shown to be susceptible.

To summarise, larvae were fed on proved infective *Arvicanthis* and nymphae from these larvae failed to transmit the virus to susceptible sheep by inoculation and to two *Arvicanthis* by feeding. The attempt to carry on infection from *Arvicanthis* to *Arvicanthis* by ticks failed.

Nymphae reared from larvae that had fed on *Arvicanthis* 54 (see Table VI) were fed on *Arvicanthis* 80 which was inoculated with virus on the day following infestation. The blood of this rat was proved infective by the inoculation of Sheep 3958 and 3999 on the day that the nymphae dropped, both of the sheep reacting and dying of Nairobi sheep disease. Two engorged nymphae, inoculated on the same day, infected Sheep 4001 with Nairobi sheep disease, demonstrating that the nymphae had been infected by feeding

Table VI. *Attempts to demonstrate infection of* Rhipicephalus simus *by feeding on* Arvicanthis.

Stage	History	*Arvicanthis* No.	Date of feeding	Result of inoculation of rat's blood	Result of inoculation of engorged ticks or feeding subsequent stage
Larvae	Bred from females which dropped from Sheep 3622 during a reaction, 5. x. 31	54	17 to 21. xii. 31	Sheep 3988, inoculated 23. xii. 31, did not react and proved susceptible	Sheep 3984 and 3985 received emulsion of 200 larvae 21. xii. 31. Did not react and proved susceptible
,,	Ditto	55	24 to 29. xii. 31	Sheep 3993 and 3994 inoculated 29. xii. 31. 3993 did not react and was proved susceptible. 3994 did not react and gave a dubious reaction on test	Sheep 3992 received emulsion of larvae 29. xii. 31. Did not react and was proved susceptible
,,	Ditto	57	20 to 25. xii. 31	—	—
,,	Bred from females which dropped from Sheep 3622 on the first day of the reaction	20	,,	—	—
,,	Bred from female fed on clean Sheep 3858	22 and 23. Inoculated 4. ii. 32 with virus	3 to 8. ii. 32	Sheep 3949, 3959 and 3966 inoculated 8. ii. 32. 3949 and 3959 reacted, 3966 did not react but proved immune	After moulting, unfed nymphs were inoculated to Sheep 4087, which did not react and proved susceptible. Forty-five of these nymphs fed on *Arvicanthis* 84, which did not become infected
Nymphae	Bred from larvae reared on *Arvicanthis* 22 and 23 (above). Supposedly infected. 32 on each rat	82 and 83	17 to 21. iii. 32	—	Last fourteen nymphae to drop inoculated to Sheep 4076 which did not react and proved susceptible
,,	Bred from larvae reared on *Arvicanthis* 20 and 57 (above). Supposedly clean, 16 and 25 on each rat	—	18 to 22. iii. 32	—	After moulting 4 males and 3 females were placed on Sheep 4125, they fed well but the sheep did not react and later proved susceptible
,,	Reared as larvae on *Arvicanthis* 54 (above)	80. Inoculated with virus	26 to 30. i. 32	Sheep 3958 and 3999 inoculated 30. i. 32. Both reacted and died	Sheep 4001 inoculated 30. i. 32 with emulsion of two nymphae reacted. Adults reared from remainder fed on Sheep 4091 and 4097 which did not react and proved susceptible

on *Arvicanthis* 80. Adults moulted from these nymphae were fed on Sheep 4091 and 4097, five on each animal. All the ticks fed well but neither of these animals reacted and both were later shown to be susceptible.

This experiment demonstrated that while it was possible to infect nymphae by feeding on infective *Arvicanthis*, the infection was lost during the moult.

In addition to experiments with laboratory bred ticks, adult *Rhipicephalus simus* reared from nymphs collected from *Arvicanthis* trapped on the infected farms were fed on Sheep 3960. They did not provoke a reaction and this sheep was later shown to be susceptible.

We were persistent in our efforts to demonstrate transmission of the virus by *Rhipicephalus simus* since experience with *Rhipicephalus appendiculatus* had shown that not every tick successfully carries on infection from stage to stage. Indeed one encounters quite inexplicable failures in which whole batches of ticks fail to transmit, while in other batches every single tick tested will prove infective. Possibly we are dealing here with a phenomenon similar to that recorded by Storey (1933) in his observations on the transmission of the virus of streak disease of maize by leaf-hoppers. The existence of an hereditary factor responsible for the production of active and inactive races would however be much more difficult to demonstrate in the case of an animal virus and a tick vector.

Rhipicephalus evertsi Neumann 1897

This was the next species examined. It is a two-host tick, and as we have already recorded the immature stages were collected from sheep and goats during the Athi Plains outbreaks. It will be recalled also that adults were numerous on sheep on the infected farms in Laikipia. In certain cases larvae and nymphae hatched in the laboratory were fed on sheep with the object of infecting them and the reaction provoked by inoculation was timed to take place either during the larval feeding or during the subsequent nymphal feeding. Whether the ticks were infected as nymphae or larvae the adults in several tests failed equally to infect known susceptible sheep. In another experiment after completion of the larval feed, the larvae were removed from their host at death from Nairobi sheep disease. In spite of this treatment they moulted to nymphae at room temperature and fed well as nymphae on a susceptible sheep without provoking a reaction. It may be of interest to record that these nymphae eventually moulted to adults and were fed.

We succeeded in infecting adults by feeding them on sheep reacting to inoculation of virus. The infectivity of the meal was controlled by the inoculation of some of the engorged *R. evertsi*, and by parallel feeding of *R. appendiculatus* on the other ear. In no case however did the larvae reared from these adults transmit the virus to susceptible sheep.

In two other experiments sheep were inoculated with virus while the nymphal feed was in progress, and the infectivity of these engorged nymphae was proved by the inoculation of susceptible sheep; but in neither case did

Table VII. *Attempts to transmit with* R. evertsi.

Stage	History	Sheep No.	Result	Immunity test
Nymphae	Collected as engorged larvae at death of Sheep 3844 from the disease	3803	Small rise in temp.	Susceptible
Adults	Fed during larval and nymphal stages on Sheep 3779 which reacted during the larval feed	3822	No reaction	,,
,,	Ditto	3824	,,	,,
,,	Ditto	3818	,,	,,
,,	Fed during larval and nymphal stages on Sheep 3852 which reacted during the nymphal feed	3809	Short rise in temp.	,,
Larvae	Bred from female collected at death from a natural case of the disease	3834	No reaction	,,
,,	Bred from female which dropped from Sheep 3824 at height of reaction	3827	,,	,,
,,	Bred from female which dropped from Sheep 3822 three days after reaction	3819	,,	,,
,,	Bred from female which was collected from Sheep 3954 at death from the disease*	4081	,,	,,
,,	Ditto	4088	,,	,,

* A number of the males and females collected from Sheep 3954 were emulsified and inoculated to Sheep 3986 to prove that infection had taken place. This sheep reacted and died of Nairobi sheep disease as a result of the inoculation.

adult ticks reared from these infected nymphae transmit the virus to susceptible sheep.

Rhipicephalus pulchellus (Gerstaecker 1873)

Nymphae and adults of *R. pulchellus* were collected from sheep in both the Laikipia and the Athi Plains outbreaks. In view of the wide distribution of this species in areas where Nairobi sheep disease is either absent or extremely uncommon it was not anticipated that this tick would prove to be capable of vecting the virus. A few tests made with different stages tend to confirm this view. Incidentally we experienced no difficulty in feeding any of the stages of this species on sheep.

Adult *R. pulchellus* that had been collected as nymphae from a natural case of the disease on the Athi Plains were fed on a suceptible sheep, No. 3956, and did not provoke any reaction. Nymphae bred in the laboratory were fed on Sheep 3964 during a reaction provoked by the inoculation of virulent blood, and simultaneously nymphae of *R. appendiculatus* were fed on the other ear. The engorged nymphae of *R. appendiculatus*, after moulting to adults, provoked a typical reaction in Sheep 4094, which died of Nairobi sheep disease. Some of the engorged nymphae of *R. pulchellus* were inoculated twenty-five days after dropping into Sheep 4075, which did not react, while others, after moulting to adults, were fed on Sheep 4098, which also did not react and was later shown

to have been susceptible. In a duplicate experiment with nymphae of *R. pulchellus* fed on Sheep 4074 during a reaction to inoculated virus, thirty-five engorged nymphae failed to transmit to Sheep 4074 by inoculation twenty-five days after engorgement. Similarly after moulting ticks of this batch failed to transmit when allowed to feed on Sheep 4099, which was susceptible.

Rhipicephalus bursa Canestrini and Fanzago 1877

Although *R. bursa* could not be collected from sheep in the Laikipia outbreak a few adults and two nymphae of this species were collected from affected sheep on the Athi Plains. The two nymphae moulted to female adults and were placed with two laboratory reared males on Sheep 3946 on November 4th, 1931. One female commenced to feed immediately. Sheep 3946 reacted on the third day of the tick's feeding and died of Nairobi sheep disease (see Chart I). The

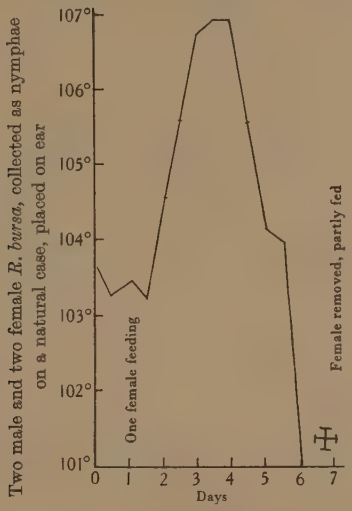

Chart I. Sheep 3946

remaining female and the two males were removed and placed on Sheep 3957. The female fed indifferently and Sheep 3957 did not react, and was later proved to be susceptible.

The few attempts that have been made to confirm transmission of the virus by *R. bursa* have been abortive, largely because we persisted in attempting to feed the larvae on sheep and so repeatedly lost our stock. In four separate attempts to feed larvae on the ears of sheep and lambs we obtained no engorgement. In the only test made, nymphae fed fairly well on the ears of an inoculated sheep; but, although the engorged nymphae were removed at the death of the host from Nairobi sheep disease on the eighth day, adults reared from these nymphae failed to transmit the virus to a susceptible sheep. In view of the single successful transmission we propose to repeat these tests.

Hyalomma aegyptium (Linn. 1758) Koch 1844

Although Nuttall (1913) and Lewis (1932) have recorded successful feeding of larvae of *Hyalomma aegyptium* on sheep, Patton (Patton and Cragg, 1913) states that in a long series of attempts he failed to induce the larvae to feed on sheep and quotes Lounsbury's experience in South Africa as being similar. Our own attempts to feed a strain of larvae on sheep in transmission experiments have failed, although it must be admitted that we have only used the ears. Possibly the larvae may feed better on the scrotum. Further tests are necessary before it can be accepted that this species cannot transmit Nairobi sheep disease; but, in so far as its host preferences in the early stages lead it to feed on hosts other than sheep and goats, its chances of functioning as a successful vector of Nairobi sheep disease are proportionally reduced.

Amblyomma variegatum (Fabricius 1794)

Clean larvae of *A. variegatum*, reared from adults that had engorged on cattle, were placed on Sheep 3971 which was inoculated with virus. The larvae fed well and dropped during the temperature reaction. Three months later nymphae moulted from these larvae were placed on the ears of Sheep 4058, 4062 and 4006. Sheep 4058 reacted typically on the fifth day of the feeding and recovered. It was immune to a subsequent test with the virus of Nairobi sheep disease. Sheep 4062 reacted also on the fifth day and was immune to a subsequent test with virus (see Chart II). Sheep 4006 did not react, but was later found to have been immune.

Engorged nymphae of the batch responsible for the transmission dropped from Sheep 4062 during and after the reaction. Adults reared from the nymphae that had dropped during the reaction were fed on Sheep 4364 three months later. This animal reacted on the sixth day of the feeding and recovered. He was later proved immune. Adults derived from nymphae that dropped from Sheep 4062 four, five, and six days after the finish of the reaction were fed on Sheep 4054. The adults were slow in beginning to feed, probably because the male is not attractive to the female until it has fed for some three days and the female does not settle down to feed until fertilised. However, twelve days after the ticks were placed on the ear, Sheep 4054 reacted and recovered (see Chart III). The nature of these reactions was proved in each case by the inoculation of susceptible and immune sheep.

The transmission from Sheep 4062 is a further example of the possibility of infection being carried on by reinfection of the transmitting ticks, if they remain attached until the host animal reacts and the blood becomes infected. In this instance ticks which completed their meal some days after the expiry of the period during which the blood is normally infected nevertheless obtained, presumably earlier in their feeding, sufficient virus to bring about a fresh infection of the gut.

As yet we have been unable to demonstrate transmission of the virus from

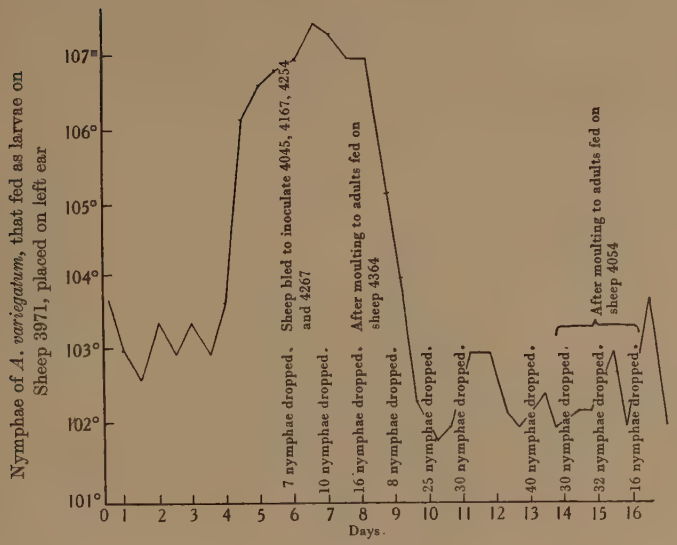

Chart II. Sheep 4062

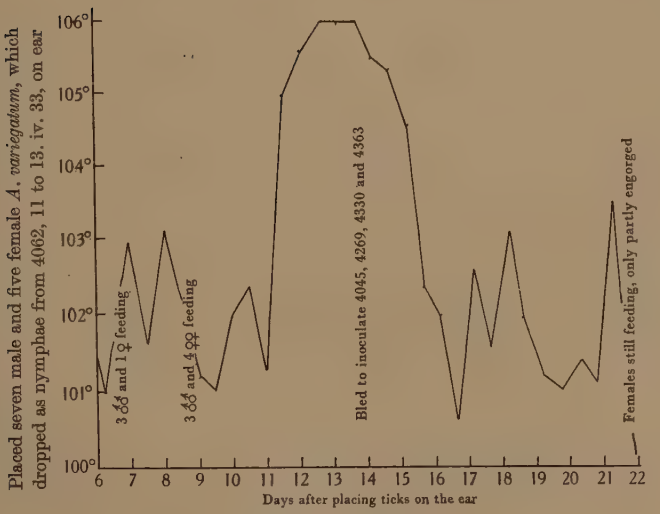

Chart III. Sheep 4054

an infected female through the egg to the ensuing larval stage. In part this has been due to the difficulty of timing reactions to coincide with the final engorgement of the *Amblyomma* females; but there is also little doubt that *A. variegatum* is not a highly efficient transmitter of Nairobi sheep disease, and parallel to the successful experiments we could record a number of failures to transmit with ticks taken from the same lots.

Discussion

It has been shown that extensive outbreaks of Nairobi sheep disease giving rise to heavy mortality have occurred in localities from which the normal vector, *Rhipicephalus appendiculatus*, is absent. It has been our general impression that such outbreaks have shown less tendency to rapid spread and that the lesions in individual cases have been less acute in character than in the ordinary outbreaks of Nairobi sheep disease transmitted by *R. appendiculatus*. The two outbreaks of disease investigated occurred in areas more than 120 miles apart. Of the tick species common to both areas, every one has been submitted to experimental tests of its capacity to vect the virus, and so far only *Amblyomma variegatum* has been proved to transmit. With this species it has been possible to infect sheep by feeding nymphae that have been infected as larvae, and by feeding adults that have been infected as nymphae. We have not yet been successful in demonstrating the passage of virus from an infected female through the egg to the larvae.

In our experiments with *A. variegatum* we experienced a greater proportion of failures to transmit than is customary with *Rhipicephalus appendiculatus*, and we have tentatively concluded that *Amblyomma variegatum* is a less efficient vector than *Rhipicephalus appendiculatus*. This conclusion accords with epizootiological experience in the outbreaks referred to in the previous paragraph, and may partly explain why there are certain extensive areas, such as the Narok district (Daubney and Hudson, 1931), heavily infested with *Amblyomma variegatum* but nevertheless free from or only rarely visited by Nairobi sheep disease. Lewis (1934, in press) states that Masai in the Narok district have informed him that a disease, which he concludes is Nairobi sheep disease, decimates their sheep at intervals of seven years. If *A. variegatum* is the responsible vector in this area one would expect occasional introductions of virus from neighbouring districts infested with *Rhipicephalus appendiculatus*, which would be more or less successfully carried on by the former species. It is worthy of remark that *Amblyomma variegatum* is less closely associated than *Rhipicephalus appendiculatus* with domestic stock, particularly sheep, in its first two stages, as anyone who has systematically tried to collect larvae and nymphae from this host will be well aware.

In general the behaviour of *Amblyomma variegatum* in respect both of host relationships and of actual capacity to transmit the virus would account quite well for the peculiar features of the Laikipia and Athi Plains outbreaks and for the relative immunity of these areas and the Narok area for some years. We

have little doubt that the vector in the Laikipia outbreak was *A. variegatum* and that the disease was introduced from foci of infection in the Nyeri district where the normal vector is abundant and the disease by no means uncommon.

With regard to *Rhipicephalus bursa*, it is to be noted that this species was never collected in Laikipia and could not possibly have been functioning as vector in that outbreak. In view, however, of the single transmission effected by a female collected from an infected sheep on the Athi Plains, it is necessary to make further tests of this species as a vector. It can only be a relatively inefficient vector, since our experience indicates that only the nymphae and adults need be taken into account. While it would be unsafe to conclude that all the other species tested are unable to vect the virus, we feel that the tests with *R. simus* and *R. evertsi* have been sufficiently exhaustive to justify such a conclusion in respect of these two species.

We desire gratefully to acknowledge the valuable assistance that we have received from Dr J. I. Roberts of the Medical Research Laboratory, Nairobi, particularly in the provision of wild rodents and the collection of their ecto-parasites. We are also indebted to Dr E. A. Lewis for many determinations of ticks collected during this investigation.

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TWO NEW SPECIES OF COCCIDIA: *EIMERIA TRIFFITT* N.SP. OF THE ELAND (*ORIAS CANNA*), AND *EIMERIA PERUVIANA* N.SP. OF THE LLAMA (*LAMA GLAMA*)

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(With 3 Figures in the Text)

Eimeria triffitt n.sp. (Fig. 1)

The faeces of five elands (*Orias canna* syn. *Taurotragus oryx*) from Ascania Nova (Ukraine) were examined after concentration by Darling's method. Coccidial oocysts were found in the faeces of one of the animals. The oocysts were 21–24μ in length and 15–19μ in breadth, with an average size of 21·1 × 17·8μ. The largest measured 24 × 19μ and the smallest 21 × 15μ. The shape-index was 1 : 0·71–0·85, with an average of 1 : 0·85.

When kept in 2·5 per cent. solution of potassium bichromate four spores developed inside the oocyst. The spores had rounded ends and measured 9 × 4·5–6μ. The sporozoites were pyriform with one rounded and one pointed end. No residual body was present in the oocysts, and none was clearly visible in the sporocysts.

The first recorded coccidian from an eland was *Eimeria canna* described by Triffitt (1924). This species has oval oocysts with a triple membrane and a micropyle at one end. The oocysts measure 23·5–34 × 16·5–20μ, the sporoblasts 7–10·5μ and the oval spores 12–16·5 × 5·5–6·5μ. The sporozoites are pyriform with a visible nucleus (1–1·4μ). Residual bodies are present in the sporocysts, and there is sometimes an irregular granular residual mass in the oocyst.

The coccidian described in the present paper differs from *Eimeria canna* in the absence of a micropyle, the absence of a residual body in the oocyst, and also in its smaller size. It is proposed to name it *Eimeria triffitt* in honour of Miss M. J. Triffitt who was the first to describe a coccidian from the eland.

Eimeria peruviana n.sp. (Figs. 2 and 3)

As yet there has been no published record of a coccidian from the llama. The faeces of a number of animals were sent to me for examination by Prof. M. F. Iwanoff, and amongst them samples from one male and four female llamas (*Lama glama*). After concentration by the method of Darling coccidial oocysts were found in the faeces of one of the females.

The oocysts were oval with a double membrane. It seemed as though one of the specimens had a micropyle whereas the others were devoid of one. The

protoplasm of the non-sporulated oocysts was reduced to a spherical mass. The size of the oocysts was 27·9–37·5 × 18–22·5 μ, with an average of 31·8 × 19·3 μ. The maximum size was 37·5 × 22·5 μ, and the minimum 27·9 × 18 μ. The shape-index was 1 : 0·60–0·70, with an average of 1 : 0·61. Four spores each with two sporozoites developed inside the oocysts: the size of the spores was 10·5–15 × 7·5 μ. A large residual mass was always present in the centre of the oocyst.

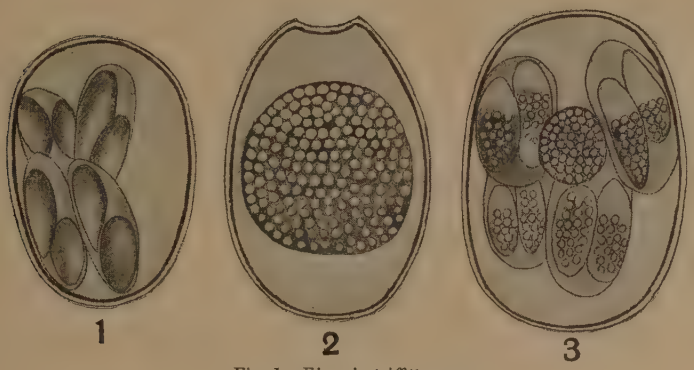

Fig. 1. *Eimeria triffitt* n.sp.
Figs. 2 and 3. *Eimeria peruviana* n.sp.

In considering whether this coccidian is a new species it must be compared with the coccidian of the camel, an animal nearly allied to the llama. *Eimeria cameli* of the camel was discovered by Miss P. S. Iwanoff-Gobzen (1932) in Kasaktan (Russia). The oocysts of this species are oval and their size, according to our measurements, 21–36 × 18–27 μ, with an average of 28·7 × 20·3 μ. The shape-index is 1 : 0·62–0·90, with an average of 1 : 0·76. Residual bodies are present in the sporocysts but not in the oocysts.

The coccidians of the camel and the llama therefore belong to different species, and we propose for the latter the name *Eimeria peruviana* n.sp.

Our thanks are due to Prof. M. F. Iwanoff who sent us the material.

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THE SUSCEPTIBILITY OF AUTOGENOUS AND ANAUTOGENOUS RACES OF *CULEX PIPIENS* TO INFECTION WITH AVIAN MALARIA (*PLASMODIUM RELICTUM*)

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CONTENTS

Introduction

In Europe and North America *Culex pipiens* L. appears to be the common vector of *Plasmodium relictum* of birds, but different authors have published divergent results as regards the infection rate obtained in *C. pipiens* with this parasite. Thus Et. and Ed. Sergent (1921), working in Algiers, found that when *C. pipiens* fed on birds with suspected latent infections of *P. relictum* they became infected in 26 out of the 35 lots which were tested. That is to say, malarial infections which were of such low grade as not to be revealed by direct microscopical examination produced infection in *C. pipiens*. These authors, however, do not give figures for the rate of infection produced in the mosquitoes when they were fed on either chronic or acute *P. relictum* infections.

Huff (1927, 1929), working in America, obtained by selection races of *C. pipiens* susceptible and non-susceptible to infection with *P. cathemerium*; and later (1931) showed that selection had a similar influence on *C. quinquefasciatus*, and that in *C. pipiens* susceptibility behaved as a simple recessive character. Huff (1930), by "double infectious feedings" experiments, showed that susceptibility and non-susceptibility of *C. pipiens* to infection with species

of *Plasmodium* is specific for the parasite. The average infection rates obtained in *C. pipiens* were: *P. cathemerium* 26·9 per cent.; *P. elongatum* 3·2 per cent.; and *P. relictum*, two strains, 89·4 and 87·3 per cent. respectively. In a more recent paper, Huff (1932) gives 88 per cent. as the infection rate produced in *C. pipiens* by *P. relictum*, and says that *P. relictum* has a greater infectivity rate for *C. pipiens* than *P. cathemerium*, of which the infection rate is given as 35·7 per cent.

Kikuth and Giovannola (1933), on the contrary, state that they obtained a much higher infection rate in *C. pipiens* with *P. cathemerium* than with *P. relictum*, and that transmission to birds is much more regular with mosquitoes infected with *P. cathemerium* than with those infected with *P. relictum*. They obtained 100 per cent. successful transmissions with *P. cathemerium* infections compared with only 70–80 per cent. with *P. relictum* infections. Kikuth and Giovannola also state that some individual birds are refractory to infection with sporozoites, while they have never come across a case of resistance to blood inoculation amongst many thousands of inoculations; and also that there appears to be some seasonal influence on the transmission of avian malaria by *C. pipiens*, as during the summer experiments are easily carried out but during the winter some experiments fail for no apparent reason.

Reichenow (1932), with a Hamburg strain of *P. relictum*, obtained 90–100 per cent. infections in *C. pipiens*.

Roubaud (1933) says that as a result of preliminary experiments he has found that an "autogenous" Parisian race of *C. pipiens* is but feebly susceptible to infection with *P. relictum*. Tate and Vincent (1932 *b*) obtained an infection rate of 55 per cent. in English *C. pipiens* with a German strain of *P. relictum*.

ORIGIN OF THE STRAINS OF *CULEX PIPIENS* AND *PLASMODIUM RELICTUM* USED IN THE EXPERIMENTS

In the course of work on the chemotherapy of avian malaria[1] we have made infection experiments with English *C. pipiens*, both hibernating and laboratory bred, and three continental strains of autogenous *C. pipiens*[2], one from Greece, one from Hungary and one from Malta; and two strains of *P. relictum* Grassi and Feletti 1891, one, a German strain, obtained from the late Dr Roehl of Elberfeld, Germany, and the other, an Algerian strain, obtained from Prof. Ed. Sergent of the Pasteur Institute of Algiers.

[1] This work was done on behalf of the Chemotherapy Committee of the Medical Research Council, London.

[2] We agree with Roubaud (1933) (Roubaud and Toumanoff, 1930) in recognising two biological races of *C. pipiens* for which he has introduced the terms *Autogenous* and *Anautogenous*. The main characters of the autogenous race are: ability to oviposit without any food in the imaginal stage; absence of hibernation; and the capacity for fertilisation in a confined space (stenogamy). The main characters of the anautogenous race are: necessity for a blood meal before oviposition; the occurrence of hibernation; and the necessity of a large space for fertilisation (eurygamy).

The strains of *C. pipiens* originated as follows. The English *C. pipiens* were collected as hibernating females in a cool basement. After these females had been induced to gorge on canaries some of them laid eggs from which a laboratory strain of *C. pipiens* was started (Tate and Vincent, 1932 *a*). This strain was maintained breeding continuously in the laboratory for over 18 months.

The Greek strain derived from females which were collected by Dr Anton Papadakis in pig-sties in the State Agricultural College at Drama, Macedonia, and were forwarded to Cambridge by rail and air-mail in August 1932. After a blood meal some of the surviving females laid eggs and some of the imagines raised from them laid autogenous egg rafts. The autogenous strain started from these rafts has now been maintained in the laboratory for 30 generations without a blood meal.

The Hungarian strain was started from larvae collected in August 1932 at Tihany, Hungary, by one of us (M. V.). From these an autogenous strain was derived (Vincent, 1933) which has now been kept for 25 generations without a blood meal.

The Maltese strain began with 15 females collected in Malta by Dr O. Theodor, of the Hebrew University, Jerusalem, during November 1932 and sent to Cambridge by air. Eggs laid by some of the females after gorging on canaries gave rise to imagines some of which laid autogenous rafts. This strain has been kept for 27 generations without blood meals.

In addition crosses between some of these strains, Greek-English, and Greek-Hungarian, were used for infection experiments.

Methods

The birds used for the experiments were all hen canaries (*Serinus canarius*). The engorged mosquitoes were incubated in a thermostat at 24° C. and were fed on slices of apple. Biting experiments were done at room temperature, about 17° C., and the canaries to be bitten were placed in small cages inside larger cages containing the mosquitoes. They were either placed in a dark room or were left in overnight. With this method there is no necessity to remove feathers or to tie the birds. In darkness or dim light the birds remain quiet and the mosquitoes readily gorge, biting between the scales on the legs and toes. About 50 mosquitoes may gorge without the bird showing any ill-effects, and the bitten birds do not appear to suffer any discomfort from the bites. Blood films from the canaries were made from the toe and the films were stained with Leishman's stain.

Results

(*a*) *Infection of various strains of* Culex pipiens *with an Algerian strain and a German strain of* Plasmodium relictum

In these experiments all the strains of *C. pipiens*, English (hibernating, anautogenous) and Greek, Hungarian and Maltese (autogenous), were fed on

birds infected with either the German or Algerian strain of *P. relictum*. As the experiments extended over a period of 18 months, and many different lots of mosquitoes were used, it is impracticable to give details of individual experiments, and the results of those in which mosquitoes of the various strains were fed on birds with acute infections of the Algerian or German strain of *P. relictum* are tabulated in Table I. For these experiments birds were selected which showed at least two gametocytes in a three minutes' examination of a stained blood film. It may be said at this point that the two strains of malaria vary markedly in their tendency to form gametocytes. In the Algerian strain they are readily formed in most of the infected birds during at least part of the acute period, but in the German strain the development of gametocytes is capricious, and many birds may show no gametocytes in the blood throughout the acute stage of infection while others may have several gametocytes in each microscopical field. These variations may be found in a series of birds inoculated at the same time with equal amounts of the same infected blood. It is interesting that recently Huff and Gambrell (1934) have shown that there is great variation in the formation of gametocytes in *P. cathemerium*, in strains of which no gametocytes may be formed for many months.

Table I. *Infection of various strains of* Culex pipiens *with an Algerian and a German strain of* Plasmodium relictum.

Strain of *P. relictum*	Strain of *C. pipiens*	No. lots fed on infected birds	No. which gorged	Gut infections Inf./dis.*	Salivary gland infections Inf./dis.*	Total Inf./dis.*	% infected
Algerian	English	11	419	20/22	77/91	91/104	88
,,	Greek	15	483	60/77	133/157	183/209	88
,,	Maltese	11	351	18/18	59/62	59/62	95
,,	Hungarian	6	186	8/9	68/80	71/83	86
,,	Greek-Hungarian	2	63	5/5	21/21	26/26	—
,,	Greek-English	1	23	—	2/3	2/3	—
	Total	46	1525	111/131 =85 %	360/414 =86 %	432/487	89
German	English	10	314	20/46	36/102	50/123	41
,,	Greek	4	123	6/12	14/37	14/37	38
,,	Maltese	2	77	4/5	2/10	6/14	—
,,	Hungarian	1	8	3/6	—	3/6	—
,,	Greek-Hungarian	1	11	—	1/6	1/6	—
,,	Greek-English	1	54	3/5	12/24	15/29	(52)
	Total	19	587	36/74 =49 %	65/179 =36 %	89/215	43

* Inf./dis. = Infected/dissected.

From Table I it is seen that with the Algerian strain of *P. relictum* the infection rates obtained for the various strains of *C. pipiens* are: English, 88 per cent.; Greek, 88 per cent.; Maltese, 95 per cent.; and Hungarian, 86 per cent. It is evident that the variation from 86 to 95 per cent. is insignificant in such experiments, and it can be concluded that all these strains of *C. pipiens* behave similarly towards infection with the Algerian strain of *P. relictum*. The figures for the cross-bred strains are too small to be treated

separately, but their susceptibility does not appear to differ from that of the pure strains.

If the figures for the different strains of mosquitoes are added together it is found that 46 lots of mosquitoes, comprising 1525 females, engorged on infected birds. Of these females 487 were dissected and 432 had either oöcysts on the gut or sporozoites in the salivary glands. This gives an average infection rate for this strain of malaria of 89 per cent. It is also noteworthy that the percentage infection rates for gut and salivary glands are nearly equal—85 and 86 per cent. respectively.

The figures shown in Table I for the German strain are much less full, but it is shown that the infection rates in English and Greek *C. pipiens* are 41 and 38 per cent. respectively. The figures for the remaining strains, Maltese and Hungarian, and for the cross-bred strains, are too small to be considered separately except that they indicate no significant difference between them as regards susceptibility to this strain of *P. relictum*. When the figures are totalled it is found that 19 lots of mosquitoes comprising 587 females were fed on infected birds. 215 females were dissected and 89 of them were infected, giving an average infection rate of 43 per cent. for the German strain of *P. relictum*. It is also noticeable that with this strain the average infection rate for guts is higher than for salivary glands, 49 and 36 per cent. respectively.

We found that if hibernating females were subjected to prolonged illumination and thereby induced to gorge on infected birds they showed no difference in susceptibility to malaria infection compared with non-hibernating females. It is interesting, in view of Roubaud's statement (1933, p. 60) that hibernating females of *Culex pipiens* usually require several blood meals after reactivation for oviposition, that, in our experiments, oviposition followed the first blood meal as readily in the reactivated as in the non-hibernating females. For example, English hibernating *C. pipiens* females were collected on September 23rd, 1932, and were kept at laboratory temperature and illuminated at night until October 13th, when 23 of them gorged on a canary infected with the Algerian strain of *P. relictum*. Gametocytes were present in blood films at the rate of one in ten microscopical fields. At intervals 9 of the 23 engorged females were dissected and all of them had oöcysts on the gut or sporozoites in the salivary glands or both, and in some cases the guts were covered with cysts. Further, from the 23 females which gorged 11 large egg rafts were obtained within two weeks of the single blood meal. Similar results were obtained with females collected later in the year.

The results given in Table I show clearly that there is no significant difference between the various strains of *C. pipiens* tested, whether autogenous or anautogenous, in their susceptibility to infection with *P. relictum*. The females of the autogenous strains used derived from cultures which had been bred without blood meals for periods varying from the 2nd to the 25th generation, but no change in behaviour, as regards avidity in biting, or susceptibility to malaria, was evident between the different generations. On the other hand,

there is a very significant difference between the infection rates produced by the different strains of *P. relictum*, and it is clear that the Algerian strain produces an infection rate of about 89 per cent. irrespective of the strain of *C. pipiens*, whereas the German strain produces an infection rate of only about 43 per cent. irrespective of the strain of *C. pipiens*.

(b) The relation of the number of gametocytes to the infection rate

In these experiments the numbers of gametocytes present in the blood of the bitten birds varied from two in a three minutes' examination to several in each microscopical field, but as similar variations occurred in both strains of malaria and many different lots of mosquitoes were used the number of gametocytes would not alone account for the difference in infection rates obtained for the two strains of malaria. The simplest way to examine the relation of the number of gametocytes present to the infection rate is to tabulate the results obtained with mosquitoes fed on birds with few gametocytes and those fed on birds with more numerous gametocytes and to compare the infection rates in the two groups. It is convenient to regard less than 10 in three minutes' examination as few gametocytes and 10 or more as more numerous gametocytes. The results are tabulated in this manner in Table II.

Table II. *Relation of number of gametocytes to infection rate in* Culex pipiens.

Strain of *P. relictum*	Strain of *C. pipiens* infected	Number of gametocytes in blood: Fewer than 10 in 3 min. Pos./dis.*	Number of gametocytes in blood: 10 or more in 3 min. Pos./dis.*
Algerian	English	63/75	87/91
,,	Greek	72/86	114/118
,,	Maltese	9/9	42/45
,,	Hungarian	—	71/83
	Total	144/170 =85 %	314/337 =93 %
German	English	25/75	85/124
,,	Greek	3/15	6/13
,,	Maltese	—	6/14
,,	Hungarian	—	3/6
	Total	28/90 =31 %	100/157 =64 %

* Pos./dis. = Positive/dissected.

Table II shows that in the Algerian strain of malaria there is no significant difference in the infection rate produced in mosquitoes fed on birds with relatively few gametocytes and those fed on birds with more numerous gametocytes, the infection rates being 85 and 93 per cent. respectively. With the German strain, however, there is a marked difference in the infection rates in the two groups, as the rate for the group with fewer gametocytes is 31 per cent. and that for the other group 64 per cent. It is also noticeable, in comparing the two strains of malaria, that the infection rate produced by the first group (few gametocytes) with the Algerian strain is higher than that

produced by the second group (more numerous gametocytes) with the German strain.

It appears, therefore, that with the Algerian strain of *P. relictum*, provided gametocytes are found to be present by direct examination of the blood, the actual number has little or no influence on the infection rate produced in *C. pipiens*; but with the German strain there is a marked increase in the infection rate of mosquitoes fed on birds showing relatively numerous gametocytes compared with those fed on birds with comparatively few gametocytes. No attempt was made to record differential counts of male and female gametocytes, and the smallness of the actual numbers of gametocytes present in the blood, even of birds with relatively more numerous gametocytes, made it impracticable to determine if the number of exflagellating male gametocytes (James, 1931) was the limiting factor in the infection rate produced by the German strain of malaria.

(*c*) *The intensity of the infection produced in* Culex pipiens *by the two strains of* Plasmodium relictum

There was no noticeable difference in the intensity of the infection produced in different strains of mosquitoes with the same strain of *P. relictum*; but the general intensity of the infection produced by the two strains of *P. relictum* was different. The Algerian strain nearly always produced very heavy infections. Frequently the guts were covered with oöcysts and 200–300 mature oöcysts were counted in many cases. The salivary gland infections were usually correspondingly intense. With the German strain of *P. relictum*, on the other hand, the infections produced were generally much less intense, and, although very heavy infections occurred occasionally, usually the number of oöcysts counted on the guts was not more than about 50.

(*d*) *Infection of* Culex pipiens *fed on chronic cases of* Plasmodium relictum

Several lots of *C. pipiens* were allowed to gorge on birds with chronic infections of the Algerian strain of *P. relictum* with the results shown in Table III.

Table III. *Infection of* Culex pipiens *fed on chronic cases of* Plasmodium relictum.

Strain of *C. pipiens*	Duration of malarial infection in birds bitten (months)	Result of microscopical examination of blood	No. which engorged	No. Pos./dis.
Greek	2–34	0	14	0/14
Maltese	16	1 gametocyte in 10 min. examination	24	2/20
Greek	14	0	23	13/19
		Total	61	15/53 = 28 %

These results confirm those of Et. and Ed. Sergent (1921) that *C. pipiens* may become infected by feeding on birds with chronic or latent infections of the

Algerian strain of *P. relictum* even if no parasites are found in the blood by direct examination. So far infection of mosquitoes has not resulted from feeding them on birds with chronic infections of the German strain of *P. relictum*, but the numbers dealt with are insufficient to be conclusive. Further experiments on this point are now in progress.

(*e*) *The influence of season on the infection rate in* Culex pipiens

To judge if there is any seasonal influence shown in our experiments the results of infection experiments with both strains of malaria are tabulated in Table IV according to the month in which the birds were bitten. Most of our experiments were done during the winter, or at least the colder months of the year—October–March—but sufficient were done in the warmer months—April–September—to reveal any seasonal effect.

For the Algerian strain, if the results for the colder months (October–March) are totalled and compared with the totals for the warmer months of the year (April–August, no mosquitoes having been fed in September), it is seen that although there are variations from month to month no definite seasonal effect is shown. The average infection rate for the colder months is 91 per cent. and that for the warmer months 86 per cent.

The figures for the German strain are less complete, but nevertheless they give no indication of a definite seasonal influence. The average infection rate for the colder months is 49 per cent. and that for the warmer months 52 per cent.

Table IV. *Influence of season on the infection rate in* Culex pipiens.

Strain of malaria	Month	No. females which gorged	No. Pos./dis.	% positive
Algerian	October	181	80/86	93
,,	November	353	114/125	91
,,	December	38	6/9	—
,,	January	185	45/52	87
,,	February	169	55/56	98
,,	March	182	50/57	88
,,	October–March	1108	350/385	91
,,	April	243	51/53	96
,,	May	170	61/76	80
,,	June	60	11/11	—
,,	July	47	18/20	—
,,	August	85	9/14	—
,,	April–August	605	150/174	86
German	November	51	4/33	—
,,	December	70	24/44	55
,,	February	228	48/82	59
,,	March	52	7/11	—
,,	November–March	401	83/170	49
,,	May	187	46/100	46
,,	June	109	20/26	—
,,	July	33	3/6	—
,,	May–July	329	69/132	52

It is therefore clear that in our experiments the season had no influence on the infection rates produced by either the Algerian or German strains of *P. relictum*.

(*f*) *Transmission of* Plasmodium relictum *by* Culex pipiens

All the strains of *C. pipiens* which were tested proved to be capable of transmitting malaria to healthy birds. The Algerian strain was successfully transmitted by English, Greek, Maltese, Hungarian and Greek-Hungarian *C. pipiens*. During this series of experiments 59 birds were bitten by infected mosquitoes and of them 53, or 90 per cent., developed malaria.

Owing to the uncertainty of gametocyte formation fewer experiments could be done with the German strain, but it was successfully transmitted by English, Greek, Maltese and Greek-English *C. pipiens*. Seventeen of the 19 birds bitten by infected mosquitoes developed malaria, which indicates that the percentage transmission is in the region of 90 per cent. In all cases where the birds failed to become infected, with either the Algerian or German strains of malaria, they had been bitten by only one mosquito or by several which had very few sporozoites in the salivary glands.

Discussion

The foregoing results reconcile some of the divergent results of previous workers on the infection rate produced in *C. pipiens* by *P. relictum*. Our results with the Algerian strain of malaria agree with those of Huff (1932) and Reichenow (1932) that the infection rate in *C. pipiens* is about 90 per cent. On the other hand, our results with the German strain agree with those of Kikuth and Giovannola (1933) that this strain produces only a low infection rate in *C. pipiens* (as our strain originated from Elberfeld it is presumably the same strain as that used by Kikuth and Giovannola). We obtained only about 43 per cent. infection rate with this strain. It is therefore clear that two strains of the same species of avian malaria may produce very different infection rates in mosquitoes. Our results also indicate that this difference is not merely one of the relative numbers of gametocytes formed by different strains.

The work of Et. and Ed. Sergent (1921), showing that *C. pipiens* may be infected by feeding on some birds in the chronic stage of infection, is confirmed by us for the Algerian strain of *P. relictum*, but, so far, not for the German strain.

We cannot confirm the suggestion of Roubaud (1933) that autogenous strains of *C. pipiens* are but little susceptible to infection with *P. relictum*. On the contrary, no evidence of a difference in susceptibility was found in any of the strains of *C. pipiens* tested, including English anautogenous, and Greek, Maltese, Hungarian and cross-bred autogenous strains. All the strains became equally readily infected and were able to transmit the malaria to healthy birds.

Our results support those of Kikuth and Giovannola (1933) in that some birds resist infection with sporozoites of *P. relictum* (especially if bitten by one or few slightly infected mosquitoes), but we obtained a higher rate of successful transmissions, 90 per cent. compared with 70–80 per cent. which they obtained. However, the number of birds used in our experiments with the German strain was too small to be relied on for the transmission rate for this

strain of malaria. We might mention here that our experience is contrary to that of Kikuth and Giovannola, who state that they never had a case of failure to infect birds by blood inoculation. Our experience is that a very small number, less than 1 per cent., of birds may resist blood inoculation (Tate and Vincent, 1933) and on this point we agree with Et. and Ed. Sergent (1921) and Fourneau and co-workers (1930). No evidence of a seasonal effect on the transmission of malaria by *C. pipiens* such as that mentioned by Kikuth and Giovannola was met with in the course of the present work.

SUMMARY

1. Anautogenous English and autogenous Greek, Hungarian, Maltese and cross-bred strains of *C. pipiens* were infected with an Algerian and a German strain of *P. relictum*.

2. The different strains of *C. pipiens* behaved similarly as regards susceptibility and transmission towards the same strain of *P. relictum*.

3. The two strains of malaria differ in the infection rate produced in mosquitoes. The Algerian strain produces an infection rate of about 89 per cent., while that of the German strain is only about 43 per cent.

4. The difference in the infection rates of the two strains is not due merely to differences in the number of gametocytes formed.

5. In general *C. pipiens* fed on birds infected with the Algerian strain of malaria became more heavily infected than those fed on birds infected with the German strain.

6. *C. pipiens* were infected in some cases by feeding on birds with chronic infections of the Algerian strain of *P. relictum*, but not when fed on birds with chronic infections of the German strain.

7. No seasonal influence was found as regards the infection of *C. pipiens* by *P. relictum*.

8. The Algerian strain of malaria was successfully transmitted by English, Greek, Hungarian, Maltese and Greek-Hungarian strains of *C. pipiens*, and the German strain of malaria by English, Greek, Maltese and Greek-English strains of *C. pipiens*.

We wish to express our gratitude to Dr Anton Papadakis and Dr O. Theodor for their great kindness in sending us the strains of *C. pipiens* from Macedonia and Malta; and also to Prof. D. Keilin under whose direction the work was done.

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THE ACTION OF ATEBRIN ON BIRD MALARIA

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CONTENTS

Introduction

The first tests of the synthetic anti-malarial drug atebrin were made by Kikuth (1932) on canaries infected with *Plasmodium relictum*, and on Java sparrows infected with *Haemoproteus orizivorae*. Using the method of Roehl (1926) he found that atebrin had a chemotherapeutic index of 1 : 30 when employed in infections due to *Plasmodium relictum*. The chemotherapeutic index of atebrin is the same as that of plasmoquine, but using the strongest doses it does not produce such a long delay in the appearance of parasites in the blood, and it never sterilises the bird of parasites. Kikuth investigated the action of plasmoquine, quinine and atebrin at different stages in the life cycle of *Haemoproteus* and found that atebrin and quinine, in contrast to plasmoquine, do not act on the gametocytes. If infected Java sparrows are treated with plasmoquine the gametocytes disappear, only to reappear later, while the schizonts are unaffected. Birds treated with a combination of plasmoquine and atebrin, however, do not relapse, or only do so much later, proving that atebrin destroys the schizonts. Manwell (1933) tested the action of atebrin on canaries infected with *Plasmodium rouxi*, *P. circumflexum* and *P. cathemerium*, and found that while it prevents infections with *P. rouxi*, it neither prevents nor cures infections with the other two species. He tested atebrin only on birds infected by blood inoculation and not on mosquito-infected cases.

Recently the action of atebrin on sporozoite infections of birds was investigated by Kikuth and Giovannola (1933). They used two species of avian malaria, *P. cathemerium* and *P. relictum*, and came to the conclusion that atebrin, in contrast to quinine and plasmoquine, acts as a clinical prophylactic in delaying the onset of the malarial attack, and in mitigating its severity. It does not, however, destroy all the sporozoites and is not therefore a true causal

prophylactic. They attribute the prophylactic action of atebrin to its very slow elimination from the body, a fact which has been demonstrated by Hecht (1933) and by other workers.

Atebrin is now widely used in the treatment of human malaria, and extensive trials have been made on naturally acquired malaria in the tropics, and on malaria induced in hospitals for the purpose of malaria-therapy. Its action on human malaria is similar to that on avian malaria; it has a very marked schizonticidal action, but has little or no effect on gametocytes. The prophylactic action of atebrin in benign tertian malaria has been investigated by James (1933), who found that, when given shortly before the bites of infected mosquitoes and for the five following days, it delayed the onset of the primary attack in the five patients treated.

The object of the present work was to investigate the prophylactic action of atebrin in blood-inoculated, and in mosquito-induced, infections of *P. relictum* Grassi and Feletti 1891 in canaries (*Serinus canarius*).

Methods

The technique employed for the inoculation of the birds, the administration of the drugs, and the examination of the blood has already been described in a previous paper (Tate and Vincent, 1933 *b*). The atebrin to be tested was made up in distilled water from the atebrin tablets of Bayer and administered orally. The maximum dose which was tolerated for several consecutive days was 5 mg. per 20 g. body weight of bird, and this was given in 0·50 c.c. of water. Kikuth and Giovannola (1933) in some of their experiments used a dosage of 10 mg. per 20 g. body weight of bird, but they found that it was not well tolerated and in some cases they had to discontinue treatment. In all our mosquito-infection experiments we used a uniform dosage of 5 mg. and only varied the duration of the course of treatment. For this work we have used two strains of *Plasmodium relictum*—strain A which we obtained from Algiers through the kindness of Prof. Sergent, and strain G which was given to us in 1928 by the late Dr Roehl of Elberfeld. Most of the experiments were done with strain A which produces a much higher infection rate in mosquitoes (Tate and Vincent, 1934). We used *Culex pipiens* L. as the vector in all our experiments, and transmission of the malaria was always done by means of the mosquito bite, and not by injection of a sporozoite-saline mixture as employed by Kikuth and Giovannola (1933) in most of their experiments. In all cases the mosquitoes which had fed were dissected to ascertain whether sporozoites were present in the salivary glands. In only a few cases do birds bitten by infected mosquitoes fail to develop malaria. We have found that in strain A parasites usually appear in the blood about the sixth day after the infective bite, but the incubation period varies from 4 to 9 days. In strain G the incubation period is less regular and varies from 5 to 16 days.

RESULTS

Details of the action of atebrin on blood-inoculated malaria of both strains are given in Tables I and II. The first dose was given 4 hours after inoculation and five doses were given subsequently at 24-hour intervals. With both strains of malaria atebrin had a very marked action in retarding the appearance of parasites in the blood, which in the four control birds appeared on the fifth or sixth day after inoculation. The maximum tolerated dose of 5 mg. was given to one bird (1315) infected with strain G, and to four birds (1484, 1485, 1487,

Table I. *Atebrin given to birds infected by blood inoculation with malaria strain* G.

Bird no.	Atebrin after infection	Day parasites appeared in blood	Nature of malarial attack
1315	6 × 5	28	Mild
1317	6 × 0·33	13	Very mild
1316	Control	5	Heavy
1318	Control	5	Mild

Table II. *Atebrin given to birds infected by blood inoculation with malaria strain* A.

Bird no.	Atebrin after infection	Day parasites appeared in blood	Nature of malarial attack
1484	6 × 5	19	Very mild
1485	6 × 5	20	Heavy
1487	6 × 5	30	Very mild
1488	6 × 5	28	Mild
1486	Control	6	Heavy
1489	Control	5	Heavy

Explanation of Tables I and II

The dosage of atebrin is calculated in mg. per 20 g. of body weight of bird. The figures 6 × 5 in the second column mean that a dose of 5 mg. was given on the day of inoculation and on the five following days. The first dose was given 4 hours after inoculation and the subsequent doses at 24-hour intervals. In the last column *very mild* means that the number of parasites in a blood film was less than one per microscopical field at the height of infection; *mild* means that at the height of infection there were one to five parasites per microscopical field; and *heavy* means that there were more than five parasites per microscopical field at the height of infection.

1488) infected with strain A, and the incubation period in these birds varied from 19 to 30 days. A smaller dose of 0·33 mg. was tried in one case (1317), and it delayed the appearance of parasites until the thirteenth day.

The results of the tests of atebrin on mosquito-induced malaria of both strains, which are summarised in Tables III and IV, are in striking contrast to the results of the tests on blood-inoculated malaria. A total of thirty-one birds was used in the mosquito-infection experiments and parasites appeared in the blood of all but two of them, both treated and untreated, within the normal incubation period. Of the two birds which failed to show any infection, one was an atebrin treated bird (1470) and the other a control (1825), and both

were afterwards shown to be susceptible to malaria by inoculation with infected blood.

Table III. *Atebrin given to birds infected by mosquitoes with malaria strain* A.

Bird no.	No. of infective mosquitoes by which bird was bitten	Atebrin before infection	Atebrin after infection	Day parasites appeared in blood	Nature of malarial attack
1465	4	—	6×5	7	Very mild
1467	2	—	6×5	9	Very mild
1468	3	—	6×5	7	Heavy
1470	1	5×5	—	Negative to 35*	—
1471	1	6×5	6×5	7	Mild
1472	1	6×5	6×5	8	Mild
1464	2	Control	—	7	Heavy
1466	1	Control	—	6	Mild
1482	3	Control	—	5	Heavy
1495	1	—	6×5	8	Very mild
1491	3	Control	—	7	Heavy
1493	4	Control	—	6	Mild
1531	2	4×5	10×5	6	Mild
1532	3	4×5	1×5	5	Mild
1533	2	4×5	6×5	7	Heavy
1534	8	4×5	1×5	5	Mild
1537	1	—	4×5	4	Heavy
1535	6	Control	—	4	Heavy
1536	1	Control	—	4	Heavy
1538	1	Control	—	5	Heavy
1565	2	5×5	—	7	Mild
1566	2	5×5	—	7	Mild
1575	4	Control	—	6	Mild

* Then successfully reinoculated with infected blood.

Table IV. *Atebrin given to birds infected by mosquitoes with malaria strain* G.

Bird no.	No. of infective mosquitoes by which bird was bitten	Atebrin before infection	Atebrin after infection	Day parasites appeared in blood	Nature of malarial attack
1823	5	1×5	4×5	8	Very mild
1824	2	1×5	4×5	8	Very mild
1825	4	Control	—	Negative to 35*	—
1826	6	Control	—	6	Heavy
1869	5	1×5	4×5	7	Mild
1870	5	1×5	4×5	6	Very mild
1871	6	Control	—	5	Heavy
1872	5	Control	—	5	Heavy

* Then successfully reinoculated with infected blood.

Explanation of Tables III and IV

The numbers of the birds comprising each experiment are bracketed. In each experiment all the birds were bitten at approximately the same time by mosquitoes from the same infective batch. Dosage and nature of the malarial attack are indicated as in Tables I and II.

In all cases the maximum tolerated dose of atebrin (5 mg.) was administered, and its prophylactic action was tested by giving it for a varying number of days both before and after the infective bites. Three of the birds (1470, 1565, 1566)

received the drug before infection only, ten of them (1471–2, 1531–4, 1823–4, 1869–70) both before and after infection, and five of them (1465, 1467, 1468, 1495, 1537) after infection only. The relation of the time the birds were bitten to the time of dosage with atebrin could only be determined approximately, because the birds were placed in the dark with the mosquitoes for several hours, and in three cases overnight. Most of the birds received the last pre-infection dose of atebrin about 20 hours before being bitten, but two of them (1531 and 1533) received it less than 6 hours before being bitten. Of the birds which were dosed after infection, all but three (1532, 1534 and 1537) received the first dose within 6 hours of the infective bites. Subsequent doses were given at 24-hour intervals.

Bird 1531 was given a prolonged course of atebrin treatment; it received four doses before infection, the last 4 to 6 hours before infection, and ten doses after infection, the first of them within 2 hours of the mosquito bites, but nevertheless parasites were visible in the blood on the sixth day after infection, and actually 3 days before the cessation of treatment.

Although atebrin had no action in retarding the onset of the malarial attack in birds infected with sporozoites, the microscopical aspect of the asexual parasites present during the attack was quite abnormal; they were all entirely devoid of pigment granules, and were very faintly stained by Leishman's stain. Even when the parasites first appeared as late as 7 days after discontinuance of treatment the schizonts lacked pigment granules. The blood of two of the atebrin treated birds (1468 and 1565) was tested for infectivity; the blood was taken on the eleventh day after infection, when only abnormal parasites were visible in blood films, and when inoculated into clean birds it produced normal infections with pigmented parasites.

In comparing the severity of the malarial attacks it seemed that, on the whole, the atebrin treated birds had milder attacks than the control birds of the same series; and when the spleens were examined and compared a corresponding difference was noticed. Birds 1464 and 1468 were killed on the fourteenth day after infection, and portions of their spleens were fixed in Bouin's solution, sectioned and stained. Macroscopically the spleen of the atebrin treated canary (1468) was normal in size and colour, but the spleen of the control bird (1464) was very dark in colour and much enlarged. The microscopical appearance of the two spleens was markedly different; the control bird had massive deposits of pigment in the spleen, but none was visible in the spleen of the treated bird. A similar macroscopical difference was noted between the dark enlarged spleen of bird 1872 (control) and the only slightly enlarged, normal coloured, spleen of bird 1869 (atebrin treated). Both birds were killed on the eleventh day after infection, but the atebrin treated bird had had a milder attack of malaria than the control.

The effect of dosage with atebrin when started at the height of the malarial attack is very marked. One mosquito-infected bird was given a dose of atebrin on the tenth day after infection (when parasites were numerous in the blood,

and about three gametocytes were present in every microscopical field) and further doses on the three following days. Blood films were first examined about 17 hours after the first dose and, although the gametocytes were still quite normal, the asexual parasites were vacuolated and almost devoid of pigment. On the second and third days after beginning treatment the parasite level had fallen, but normal gametocytes were still present together with some abnormal, vacuolated, small parasites, which were devoid of pigment. Treatment with atebrin did not render the gametocytes non-infective for mosquitoes as was proved by feeding some mosquitoes on the bird after the third dose; of seven of these mosquitoes which were dissected, five had an infection of either the gut or the salivary glands. Two days after discontinuing the four days' treatment very few parasites were present in the blood, but that it was still infective was proved by inoculating some of it into a clean bird, which developed a normal infection. The effects of atebrin on the microscopical appearance of the parasites of bird malaria are in agreement with the observations of James (1934) on its effects on the human parasites. He found that, in benign tertian and quartan malaria, a single dose of six tablets (0·6 g.) of atebrin caused first an aggregation of the pigment grains and then their complete disappearance. He also found that the parasites became irregular in contour, and that vacuoles appeared in the cytoplasm; the chromatin, instead of forming a compact mass, became diffuse, and was finally reduced to a few lightly staining dots.

The production of peculiar bodies, which stained with Leishman's stain, in the blood corpuscles of atebrin treated birds was invariably observed. These bodies resembled those described by us (Tate and Vincent, 1932) in canaries treated with two other chemical compounds. The bodies appeared in the blood cells within 24 hours of the first dosage with atebrin, and they persisted in diminishing numbers for about 5 days after the treatment stopped.

Discussion

The results of our experiments show that atebrin has a direct destructive action on the asexual forms of *Plasmodium relictum*. It acts prophylactically against blood-inoculated infections, delaying the onset of the attack, but does not sterilise the bird of parasites. These observations agree with those of Kikuth (1932).

Our observations that atebrin treatment does not destroy gametocytes, or render them non-infective for mosquitoes, correspond with those of Kikuth (1932), who found that it did not act on the gametocytes of *Haemoproteus*, and also with a number of observations that have been made on the failure of atebrin to destroy the gametocytes of human malaria.

The results of our experiments on the prophylactic action of atebrin in avian infections initiated by sporozoites are not in accord with those of Kikuth and Giovannola (1933). We agree with them that atebrin may possibly diminish the severity of the attack which supervenes, but we found no evidence that its

onset is retarded. In this respect we found that atebrin behaved like plasmoquine, which, according to the published accounts (Russell and Nono (1932), Tate and Vincent (1933*a*) and Kikuth and Giovannola (1933)), though very efficaceous against blood-inoculated malaria of birds, is ineffective against mosquito-borne infections. Indeed, we found that in one respect atebrin is even less effective than plasmoquine; when treatment with atebrin was continued beyond the normal incubation period, parasites appeared in the blood actually during the course of treatment, but plasmoquine, given in a similar manner, delayed the appearance of parasites until several days after treatment had ceased (Tate and Vincent, 1933*a*).

The action of atebrin in avian malaria differs from that in human malaria, because the work of James (1933) shows that it is an efficient clinical prophylactic in mosquito-induced infections of the benign tertian parasite.

It is evident from our experiments that atebrin is either not toxic for the sporozoites of *P. relictum* or else that they rapidly penetrate into some situation where they are protected from its action. It is also evident that the multiplicative stages of the parasite, which follow the injection of sporozoites, are similarly unaffected by atebrin, because parasites are sufficiently numerous to be seen in blood films as soon in birds treated during the incubation period as in the control birds.

It has been suggested by some workers (*vide Malaria Commission Report of the League of Nations*, 1933) that there exists an intermediate stage of the malarial parasite between the sporozoites and the schizonts, and that multiplication takes place inside reticulo-endothelial cells of internal organs. The fact that atebrin, which is proved to destroy schizonts, is ineffective, given during the incubation period, in retarding the onset of a malarial attack initiated by sporozoites, lends some support to the hypothesis that there is some such intermediate stage of multiplication. The appearance of parasites in the blood, actually during a course of atebrin treatment, could be explained by supposing that the parasites multiply, unaffected by it, in some cells; and that they subsequently enter erythrocytes and, circulating in the blood stream, are acted on by the atebrin, which causes degenerative changes in them. The fact that parasites are abnormal in microscopical appearance, and devoid of pigment granules, even if they are first visible in the blood several days after atebrin treatment has stopped, could be accounted for by the known very slow elimination of atebrin from the circulation.

SUMMARY

1. Atebrin given during the incubation period delays the appearance of parasites in the blood of birds infected by direct blood inoculation with *Plasmodium relictum*.

2. If given to birds infected by the bites of mosquitoes it does not retard the appearance of parasites in the blood, when given either before, or after, the

infective bites, but it has some action in diminishing the severity of the attack and the degree of splenic enlargement.

3. Parasites appear in the blood of birds, infected by sporozoites, actually during the course of atebrin treatment if this is prolonged beyond the normal incubation period.

4. When asexual parasites appear in the blood of mosquito-infected birds after atebrin treatment they are abnormal in appearance and devoid of pigment grains; gametocytes are rarely seen in the blood of such birds, but if present they have normal pigment granules.

5. Treatment with atebrin produces peculiar bodies in the blood cells of canaries.

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RESEARCHES ON THE INTESTINAL PROTOZOA OF MONKEYS AND MAN

VI. EXPERIMENTS WITH THE TRICHOMONADS OF MAN AND THE MACAQUES

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Measure not Dispatch, by the Times of Sitting, but by the Advancement of the Businesse. —F. Bacon (1625), *Essay* xxv.

CONTENTS

I. Introduction

In previous instalments of this series[1] I have given some account of my researches on certain intestinal amoebae found in men and macaques. In the present paper I record the results of various similar investigations carried out concurrently with flagellates of the genus *Trichomonas*[2].

[1] See Dobell (1928, 1931, 1933) and Dobell with Bishop (1929).

[2] Some of my findings have already been briefly noted in the annual *Reports of the Medical Research Council* for 1924–5, 1929–30, and 1930–1.

Trichomonas hominis is a well-known inhabitant of the human bowel, and needs no redescription here. The comparable forms found in monkeys of the genus *Macacus* [=*Silenus*] are not so well known, however, so I must say a few words about them before describing my experiments: but I shall purposely ignore references to "Trichomonas" previously observed in "the monkey," or "a monkey," or "monkeys," as such records obviously cannot be discussed with any profit. The earliest mention of this sort which I have found is of an organism called "*T. perronciti* [or *perroncitoi*] Castellani": but I have been unable to find any description of it, or to discover the name of the host in which it was observed. To the best of my belief this "species" has never yet been described[1].

For over a quarter of a century the trichomonads of macaques have figured in the literature of protozoology; but they have often been mentioned casually in papers dealing with other subjects, and precise information about them is therefore not easy to collect. Many records are vague, or at least inexact; but so far as I have been able to ascertain a *Trichomonas* was first definitely noticed in a macaque of known species (*M. cynomolgus*) by Noc at Saigon in 1908. He found his flagellates in a female monkey which died of dysentery supposedly due to *Balantidium*, though she was also infected with "amoebae." No description was given of the *Trichomonas*.

When I began the present work, in 1924, this note by Noc, with a few equally brief records, constituted all that was known about the trichomonads of macaques—their morphology, life-history, and distribution. Since then, however, other facts have been brought to light by various workers, and it has now been ascertained that a *Trichomonas* closely similar to *T. hominis* occurs commonly in monkeys of many species—both Catarrhine and Platyrrhine. A survey of the relevant literature now reveals that intestinal trichomonads have been reported from at least 6 species of *Macacus* [=*Silenus*]. Some of the records are doubtful, but the more certain are shown in the following synopsis:

Host	*Trichomonas* sp. recorded by
(1) *M. cynomolgus* [=*irus*]	Noc (1908)
(2) *M. sinicus* [=*radiatus*][2]	Brumpt (1909); Reichenow (1925)
(3) *M. rhesus* [=*mulatta*]	?Greig and Wells (1911); Dobell (1925, 1928); Branch and Gay (1927); Hegner and Ratcliffe (1927); Kessel (1928); da Cunha and Muniz (1929); Wenrich (1933)
(4) *M. lasiotis*	Kessel (1928)
(5) *M. nemestrinus*	Dobell (1928)
(6) *M. philippinensis*	Hegner and Chu (1930)

[1] Castellani and Chalmers (1919, p. 353) enumerate "*T. perronciti* Castellani, 1907, in monkeys suffering from diarrhoea," but give no more exact reference; and I have searched in vain for further information in Castellani's publications of the year stated. In the Stiles-Hassall *Index-Catalogue* the species is marked "dead" and "*nomen nudum*," so I am content to leave it at that for the present.

[2] Owing to a clerical error, it is recorded by Bishop (1931, Table I, p. 135) that I myself found *Trichomonas* in *M. sinicus*. This is not so: for *sinicus* here read *rhesus*.

The most extensive series of macaques hitherto examined for *Trichomonas* appears to be that of Greig and Wells (1911), whose findings were recorded incidentally in a report on dysentery in Bombay. Unfortunately the species of *Macacus* studied was undetermined[1]: but from their published protocols[2] it can be gathered that these workers carefully examined 47 monkeys, in all, and found *Trichomonas* in 38—or roughly 80 per cent. The 9 "uninfected" individuals in this series were not specially studied for flagellates, however, so the observed incidence of infection was certainly minimal. The trichomonad itself was neither described nor figured.

In the present investigations I have concerned myself with the trichomonads of the Macaques and Man only: but I may note in passing, since they serve to bridge the systematic gap between these hosts, that closely similar (? identical) species of *Trichomonas* have also been found in the Anthropoid Apes—the Orang-utan (Prowazek, 1912; Mello, 1923), Chimpanzees (Deschiens, 1927; Hegner, 1934 *a*), and a Gibbon, *Hylobates hoolock* (Das Gupta, 1933).

My chief object has been to discover whether the trichomonads of men and macaques are, or are not, specifically identical. I have studied this problem from several angles, and found it more complex and difficult than it appeared originally. Most of my experiments have given results contradicting my expectations; but on reflexion I find that these contradictions really confirm one another. If the reader will read further, I think he will realize the truth of this paradox.

For the purposes of the present investigations it was necessary to study and compare the trichomonads of macaques and men in detail, and to cultivate various strains *in vitro*. At the time when I began this work, this had never been done. I succeeded, however, in isolating "pure" cultures from several species of *Macacus*, and from Man; and concomitantly other workers elsewhere seemingly had similar success. All my cultivated strains appeared, to me, to be indistinguishable specifically; and a careful comparative study of the morphology and division of one of my human and several of my simian strains made by Miss Bishop (1931) has confirmed this conclusion. No constant structural or cultural character has yet been found which enables us to discriminate systematically between the common intestinal trichomonads of macaques and human beings. I am aware, of course, that other workers do not share this opinion. Wenrich (1933), for instance, even discovered "two species of *Trichomonas*" in a single individual of *M. rhesus* which he once examined: but he has not yet published the morphological, cultural, and experimental data which can alone substantiate such a statement. In the absence of clear evidence to the contrary, I think my own scepticism is fully justified. I do not, of course, assert that all men and all macaques can or do harbour only one

[1] Recorded as "*Macacus* sp." or merely "monkeys": in all probability *M. rhesus* (and *M. sinicus*?).

[2] I have drawn my information from the data supplied in the tables dealing with both "experimental" and "normal" monkeys.

intestinal species of *Trichomonas*; but I am satisfied that the existence of more than one zoological species in these hosts has not yet been demonstrated conclusively.

As all these flagellates appear to be so closely similar, it seemed only necessary—in order to establish their identity—to prove that they are transmissible from one host to another. If they are all of one species, they should be readily inoculable from macaque to macaque, and from macaques to men and *vice versa*. But in reality such experiments are very difficult to perform, as my own attempts have shown: and unless extreme precautions are taken to obtain "clean" experimental animals, and "pure" cultures for experimentation, mistakes are almost inevitable in all work of this sort. In illustration of this truth I may note that Brumpt (1913, p. 196) once gaily remarked that *Trichomonas* is "readily inoculable from monkey to monkey, and even from monkey to pig." But he never published solid evidence for this statement—so far as I am aware—and from a more recent utterance (Brumpt, 1925) I can only conclude that he never had any. His few experiments were all insufficiently controlled, and therefore inconclusive: and he now believes apparently—on no better evidence—that *T. hominis* is not transmissible to any other mammal. It is, indeed, a fact that nobody has yet demonstrated, beyond all cavil and criticism, that the intestinal trichomonads of Man either can, or cannot, be established permanently in any other primate; nor has it previously been proved that the *Trichomonas* of any monkey whatsoever can live enduringly in any other monkey or in any human being.

Careful investigation of such problems is obviously needed, and the following pages embody the results of my own inquiries. They are published as a contribution towards the ultimate solution of several riddles—not as a final answer to them all. All my work has been done, by design, on a small scale, and performed slowly and cautiously. I am therefore able to record the results of only 14 more or less completed experiments carried out during the last 10 years. Statistically they are negligible. My excuse is that—in our present state of ignorance—I attach more importance to the quality than to the quantity of such experiments, and have been slow not from choice but from necessity.

While studying the intestinal trichomonads of macaques I had an unexpected opportunity of testing the relationship of these flagellates to those living in the genitalia ("*T. vaginalis*"). This chance I seized, and I now take this occasion to report my observations.

In concluding this brief explanatory preface, I gratefully acknowledge a debt to two of my colleagues—Miss Ann Bishop, D.Sc., and Dr P. P. Laidlaw, F.R.S. By maintaining *in vitro* several of my strains of *Trichomonas* on various occasions when I was otherwise engaged, they have given me material assistance in the prosecution of the present researches.

II. MATERIAL AND METHODS

Readers of the foregoing lines and of the earlier instalments of these *Researches* will not need to be told that my present materials have been few, and my methods as direct and simple as possible. My plan has been, throughout, to make a minimum of experiments, but to make each with the maximum of accuracy. Every experiment and observation has been made with my own hands and eyes: I have had no assistants, and rely upon the testimony of no "technicians."

To avoid, as far as possible, the obvious sources of error which have vitiated or invalidated most previous work along similar lines, I have devoted special attention to my experimental animals and to the materials used in every experiment. I inoculated no animal with trichomonads before I had studied it continuously and carefully for at least a year: and I used no species or strain of *Trichomonas* until I had isolated it in "pure" culture, and tested and examined it thoroughly. In addition I have always followed up every experiment for a period of time sufficiently long to convince myself of its outcome with complete certainty.

(1) *Experimental animals*

Five living animals—4 macaques and 1 man—have been used in the present work: but 2 other dead monkeys have contributed to it, so I must here give the essential data about all of us—3 *Macacus rhesus*, 2 *M. sinicus*, 1 *M. nemestrinus*, and myself[1].

The live monkeys were all members of my small family of tame macaques founded in 1924. Those members employed in these experiments with *Trichomonas* were[2]

(2) **Jacko** (*M. rhesus* ♂)
(3) **Mungo** (*M. sinicus* ♂)
(4) **Susanna** (*M. sinicus* ♀)
(5) **Rosa** (*M. rhesus* ♀)

All these monkeys were immature when they came into my possession, and were tamed and trained and cared for by me for the purposes of the present series of researches. They were all vigorous and healthy, but completely docile in my hands: and all of them were found—after detailed preliminary study—to be naturally uninfected with *Trichomonas*. (Evidence is given in the ensuing descriptions of the experiments.) But they were all infected with *Enteromonas*[3]—a small organism easily distinguishable from *Trichomonas*,

[1] A few particulars have already been recorded in other connexions: see Dobell (1928, 1931, 1933) and Dobell with Bishop (1929).

[2] I may remind the reader that I use the familiar old-fashioned nomenclature. By "*Macacus rhesus*" I mean the common Bengal Monkey (now often called *Macaca mulatta* or *Silenus rhesus*). "*M. sinicus*" is the Indian Bonnet-monkey (now sometimes known as *Macaca radiata* or *Silenus radiatus*).

[3] Otherwise known as *Tricercomonas* (Wenyon). Cf. Dobell and O'Connor (1921, p. 80), and Dobell (1928, p. 362).

though its presence made the search for this flagellate, in faecal specimens and in cultures, always very troublesome. The amoebic infections of these monkeys have already been noted elsewhere[1], and are of no present interest.

Jacko is now dead (killed 15. ii. 28), as also is Mungo (killed 8. viii. 28). Susanna and Rosa are still alive and in excellent health at the time of writing (June, 1934). I myself—after some half-dozen more or less successful attempts to infect myself experimentally with intestinal protozoa from monkeys—am not yet conscious of any physical deterioration.

The dead monkeys from which I obtained strains of *Trichomonas* were two female macaques (*M. rhesus* and *M. nemestrinus*), already recorded as Monkeys No. 9 and No. 10 in an earlier instalment[2]. The Rhesus monkey (No. 9) died "naturally"—*i.e.* from unknown causes—and the Pigtail (No. 10), used earlier for surgical experiments, was unintentionally killed under an anaesthetic by her owner. Neither animal had ever been used for any protozoological purpose.

As already noted, none of my experimental monkeys naturally harboured *Trichomonas*. At the times when I attempted to infect any individual, therefore, I did not usually isolate it strictly from its uninfected fellows. All these were under constant study, however, and were frequently examined, in order to ascertain whether or no they might acquire infection from the experimental animal. No such contact-infections ever occurred among my macaques. But whenever I was experimenting with two or more monkeys simultaneously, I always kept them carefully isolated until the result of each experiment was known. I took every possible precaution to guard against any accidental infections which might conceivably vitiate my findings.

(2) *The cultivation of* Trichomonas

I do not propose to discuss this subject in detail here. I shall merely mention my own methods, and record a few points necessary for the comprehension of my experiments. Since the introduction—by Boeck and Drbohlav in 1924—of the solid-liquid type of culture-medium for intestinal protozoa, the cultivation of *Trichomonas* has not been easy: it has been inevitable.

The intestinal trichomonads of men and macaques can be cultivated readily in all the varieties of Boeck-Drbohlav medium described by Laidlaw and myself[3] for cultivating *E. histolytica* and other entozoic amoebae. Primary cultures, from faeces of an infected animal, almost invariably show abundant growths of *Trichomonas* after incubation at 37° C. for 24 hours. The best medium for obtaining such initial cultures is, in my experience, that which we designated "HSre"—a slope of inspissated horse-serum, covered by egg-

[1] Dobell with Bishop (1929, p. 448), Dobell (1931, p. 8).

[2] See Dobell (1931), pp. 8–10. Particulars regarding the strains themselves will be found on another page (p. 540 *infra*).

[3] See Dobell and Laidlaw (1926), especially p. 294 (Table I).

albumin diluted with Ringer's fluid. Such medium—especially when supplemented with solid rice-starch (HSre+S)—usually gives a good primary growth not only of *Trichomonas* but also of all the other cultivable intestinal protozoa present in the inoculum. It also favours the growth of numerous intestinal bacteria and of *Blastocystis*. From a mixture of this sort, the isolation in "pure" culture (with suitable bacteria) of each several species of protozoon is often difficult: but the separation of *Trichomonas* is, as a rule, mere childsplay. These flagellates are extraordinarily hardy, and insusceptible to reactions and reagents fatal to nearly all the other intestinal protozoa of Man. They can, for example, withstand cooling to ordinary room-temperature for many days (up to about 3 weeks); an acidity equivalent to $p\mathrm{H}=4$ for several hours at least[1]; and exposure to concentrations of flavine[2] and other antiseptics which rapidly kill most starch-splitting bacteria. The trichomonads of men and macaques can also survive treatment with emetine, both *in vitro* and *in vivo*, in amounts fatal to most other intestinal protozoa[3].

Knowing these properties of *T. hominis* and its representatives in macaques —properties which I discovered gradually in the course of the present work— I now find it almost ridiculously easy to obtain "pure" cultures of *Trichomonas* from any monkey or man that I wish to study. Any primary culture made from faeces containing living trichomonads almost always shows a good growth of the flagellates in "HSre+S" medium on incubation at 37° C. for 24 hours: and by subinoculation into similar medium supplemented with flavine (1 : 10,000 or even stronger), to eradicate starch-splitting bacteria and *Blastocystis*, it almost invariably gives a good culture of *Trichomonas* only. Other flagellates or amoebae—if still present—can generally be eradicated very easily by cooling the resultant cultures for appropriate periods (10° C. for several days, or longer and at lower temperatures if necessary). Such drastic treatment usually destroys all other intestinal protozoa[4].

When once a strain of *Trichomonas* has thus been isolated in "pure" culture, with suitable bacteria, it can be propagated *ad libitum* at 37° C. It will grow readily in all varieties of Boeck-Drbohlav medium, and in many others also. Simple liquid media, composed of nothing but a little dilute serum (horse, ox, sheep, rabbit, and human have been tried, in various dilutions— usually 10 per cent. or less) are sufficient, provided the appropriate bacteria be present. I have never succeeded, however, in growing any strain of

[1] The resistance of some of my strains of *Trichomonas* to hydrochloric acid has been specially studied by Miss Bishop (1930), and I have made many similar observations myself.

[2] In a recent experiment I have found that one of my strains can even survive treatment with flavine 1 : 1000 for 30 minutes at 10° C.

[3] Unpublished experiments. I have studied a number of infected patients treated with emetine to eradicate concomitant infections with *E. histolytica*: not one has been cured of *Trichomonas* at the same time. *In vitro* this flagellate is also highly resistant to the alkaloid.

[4] *Balantidium* can often survive cooling for several days (a week or even more), and *Embadomonas* can not only live but multiply at ordinary room-temperatures (*ca.* 15° C. or higher): but these are exceptions.

Trichomonas in dilute egg-white only; though a solid-liquid medium[1] made of egg-albumin alone has been found excellent for the cultivation of several strains.

As with all other intestinal protozoa, successful and continuous cultivation depends very largely upon the concomitant bacteria. Every precaution must be taken, therefore, to avoid contamination—the strictest asepsis being necessary at all stages. "Pure" cultures of *Trichomonas*—containing an unknown admixture of bacteria as well as the flagellates—must always be treated as though really pure, if disappointments are to be avoided. Nevertheless, the ease with which good "pure" strains of trichomonads can be obtained from the most diverse initial material, shows that these protozoa are able to flourish in bacterial surroundings of great variety.

The best cultures are obtained in media which are neutral at the outset (pH = 7 to 7·2), and which remain so on incubation or develop only a slight acidity (to about pH = 6·5). Alkaline media are definitely harmful; and if—on incubation—the pH rises over 8, the trichomonads usually succumb. The presence of an organism such as *B. fecalis alcaligenes* in the bacterial flora is therefore—if present in large numbers, and not counterbalanced by acid-producing species such as *B. coli*—sometimes fatal to the continued life of a strain *in vitro*[2].

Most strains of *Trichomonas* from men and macaques ingest rice-starch with great avidity—the flagellates becoming large and bloated in consequence. Starch is not necessary, but its presence in any medium usually enhances the growth of trichomonads prodigiously. It also very greatly increases their powers of resistance to cold and harmful chemicals. For example, several of my strains which, in starch-free media, could withstand cooling to room-temperatures (*ca.* 15–20° C.) for only a fortnight or less, when fed on starch—added to the same media—could easily survive exposure to 10° C. for a period of at least a month.

With one exception all the strains of *Trichomonas* which I have studied also ingested human red blood-corpuscles, when these were added to the medium. The flagellates do not need blood for their growth, though they grow well in media made from serum, and appear to relish red-cells when they can get them.

I have now cultivated a large number of strains of *Trichomonas* for considerable periods—most of them in "HSre" medium at 37° C., in which sub-culture has usually been necessary only once a week. Some strains required more frequent transplantation, however, while others would often run for 10 days or more. But recently I have adopted a method of propagation which requires only a minimum of time and labour and culture-medium for the continuance of any strain *in vitro*: and as it may be serviceable to other workers,

[1] Slopes of heat-coagulated egg-white, covered by unheated egg-white diluted with Ringer's fluid—prepared in a similar manner to the other media of this type (Dobell and Laidlaw, 1926). This simple medium might be found useful in laboratories where serum is not easily obtainable.

[2] I have determined this by experiment: but under the ordinary conditions of cultivation, excessive production of alkali—by any organisms naturally present—only rarely causes trouble.

I may mention it here. Taking advantage of the increased resistance acquired by feeding upon rice-starch, I cultivate the flagellates in media (usually "Ehs+S") containing this carbohydrate for a couple of days or so. A rich 2-day culture, in which the trichomonads are stuffed with starch, is then removed from the incubator and cooled to room-temperature by leaving it for a few hours on the bench. I then put it in the ice-chest (*ca.* 10° C.), and leave it there for a month. At the end of this time all the flagellates are rounded-up and motionless, devoid of flagella and undulating membrane, and their ingested starch has usually been assimilated. But though many are dead, many also are alive; and by making a subculture into fresh medium, and incubating it for a day or two at 37° C., a rich growth can always be obtained. When a new generation of starch-filled trichomonads is thus procured, I return them to the ice-chest: and so on, *da capo*. With this simple procedure it is easy to keep a strain of *Trichomonas hominis* under continuous cultivation, and available for use at any moment, by devoting only 5 or 10 minutes a month to its maintenance. Strain *SVT.*—described in the next section—has been kept going in this way for over 3 years, and others for shorter periods[1], and I have no doubt that the method is capable of more general application.

I have made a large number of experiments[2] to determine how long *Trichomonas* (from men and macaques) can survive cooling to ordinary room-temperatures (*ca.* 10–20° C.). As already noted, it will usually live for a week or 10 days, and often for a fortnight or even longer: but different strains behave differently, and much evidently depends upon the concomitant bacteria, the culture-medium employed, the constancy of the temperature (frequent fluctuations being harmful), and the final *p*H of the medium. Healthy *starch-fed* flagellates will usually survive at a *constant* temperature of 10° C. for at least 1 month. But they will often live longer—this being the time which I have found to be "safe" under ordinary working conditions. How much longer some strains can remain alive I do not know. The record is held, at present, by Strain *SVT.*, which I have once recovered—apparently unhurt—after a sojourn of 47 days in the ice-chest.

Although *T. hominis* and its allies can survive at such low temperatures for so long, they neither grow nor multiply in cultures kept at ordinary room-temperatures (10–23° C.). The optimum for the development of all my strains was 37–38° C. I note this particularly because it has just been stated by Hegner (1934) that *T. hominis* is cultivable not only at room-temperatures but even at "refrigerator temperature (about 6° C.)." He gives no good evidence for this statement; and if it be true, his strains must be very different from all of mine.

[1] *Trichomonas hominis* does not, of course, form cysts: and it does not store glycogen, as most other intestinal protozoa do when they encyst. The rounded, aflagellate, and highly resistant forms—filled with starch—are, however, physiologically equivalent to cysts in their powers of endurance and resistance to cooling. The parallel is obvious.

[2] They now run into hundreds, but as the details are of no present interest it is unnecessary to describe them here in full.

(3) *Strains of* Trichomonas *used*

All the strains of *Trichomonas* used in the present experiments have been "pure" in the sense that they were cultivated without any other accompanying protozoa, though with a mixed bacterial flora suitable for their nutrition. In common with all other workers, I have hitherto failed to obtain really pure cultures of *T. hominis* (and its next of kin)—unaccompanied by any bacteria. Living bacteria appear, indeed, to be necessary for the development of these flagellates, whose food consists chiefly of such organisms.

Pure cultures—in the strict sense—are, however, really unnecessary for researches such as those about to be recorded. I have not attempted to produce a disease in any animal inoculated—only an infection with *Trichomonas*: and there is no obvious reason why the introduction of a pure culture of these flagellates into the gut of an animal, already containing a vast and entirely unknown bacterial population, should give more reliable or certain results than a like experiment made with trichomonads accompanied by other unknown intestinal bacteria derived from a similar host. If the accompanying bacteria were themselves harmful, or if they were likely to influence the experiment in any way, their presence or absence might be significant: but in the present case there is no need for such suppositions, and my results show that they may be disregarded.

In order to supply essential particulars about the cultivated strains of *Trichomonas* used in these researches, I have condensed the more important data in Table I. The reader is not asked to master all these details now; but he will find the table useful for future reference when—and if—he considers the experiments recorded in the next section.

Table I. *Particulars of the principal strains of* Trichomonas *used in the present experiments.*

Strain	Host of origin (and source)	Date of isolation	Date when abandoned*	Total period of cultivation
T.	*M. rhesus* (contents of colon at necropsy)	5. v. 25	20. xii. 28 (312)	3 years and 7½ months
FT.	Man (faeces)	25. viii. 25	8. iv. 30 (375)	4 years and 7½ months
NT.	*M. nemestrinus* (contents of colon at necropsy)	3. viii. 27	27. xii. 28 (158)	17 months
RT.	*M. rhesus*, experimentally infected with Strain *NT.* (faeces)	20. x. 28	3. iii. 30 (76)	16½ months
DT.	Man, experimentally infected with Strain *RT.* (faeces)	4. ii. 30	8. iv. 30 (16)	2 months
Δ*T.*	Ditto	7. ii. 31	25. ii. 31 (10)	18 days
ST.	*M. sinicus*, experimentally infected with Strain *DT.* (faeces)	5. iv. 30	24. iv. 30 (8)	19 days
SVT.	*M. sinicus*, experimentally infected with Strain Δ*T.* (vaginal secretion)	13. iv. 31	18. v. 34 (80)	3 years and 1 month

* The figures in parentheses denote the serial generations of subcultures.

A few notes are needed to supplement the information given in Table I. It will be seen that only 8 strains are particularly cited. These are those which I studied most carefully, but in most cases they are only representative examples. For instance, Strain *ST.* represents only one of more than a dozen similar strains investigated with equal care. Again, *DT.* and *ΔT.*—2 strains from the same host—are recorded merely because they were used for animal-experiments about to be described. Actually I have isolated and studied more than a score of similar strains from the same source during the last 4 years.

Strain *T.* was derived from the same dead monkey as Strain *T.* of *E. histolytica*—briefly described elsewhere[1]; and Strain *NT.* came likewise from a dead animal which supplied me with another strain of the same amoeba (*E. histolytica*, Strain *Nc.*)[2].

Strain *FT.* was isolated from the faeces of a man infected also with *E. histolytica*, which I likewise recovered in pure culture (Strain *F.*, already recorded[3]) and studied in detail. The patient, an Arab, was treated with emetine, which eradicated his amoebic infection but had no effect upon his *Trichomonas*. Strain *FT.* was isolated before treatment, and found—like all other strains—to be highly resistant to emetine when tested *in vitro*.

All the strains of *Trichomonas* here mentioned were morphologically indistinguishable from typical *T. hominis*[4]. Of this "species" there are, as is well known, several varieties distinguishable by the number of free anterior flagella. I must therefore record that Strain *T.* was typically provided with 3 anterior flagella ("*Tritrichomonas*"), and Strains *FT.* and *NT.* with 4 ("*Trichomonas*" or "*Tetratrichomonas*"). In cultures of every strain, however, individuals possessing 5 anterior flagella ("*Pentatrichomonas*") could generally be found. This was particularly characteristic of *NT.* and all its derivatives (*RT.*, *DT.*, *ST.*, *SVT.*, etc.). But these strains also showed occasional 3-flagellate specimens, on careful study; and in all cultures of every strain—when special search has been made for them—I have usually been able to discover individuals with only 2 anterior flagella. These anomalies are easily explicable as results of slight irregularities in the distribution and regeneration of the flagella at division, and I attach no systematic importance to them[5]. The flagella are, *on the average*, 3, 4, or 5 in number in various strains, but their number is never absolutely constant in any strain.

The morphology and method of division of Strains *FT.*, *NT.*, and *RT.* have already been well studied and described by Miss Ann Bishop (1931), so I need add nothing further here. She also made a detailed study (Bishop, 1930) of the effects of hydrochloric acid upon three strains (*T.*, *FT.*, *NT.*), and found that Strain *T.* was the least resistant. These findings will be referred to later, in another connexion[6].

[1] Cf. Dobell (1931), p. 9.
[2] Cf. Dobell (1931), p. 10 *et alibi*.
[3] Cf. Dobell and Laidlaw (1926) and Dobell (1931), p. 50.
[4] Cf. Dobell and O'Connor (1921), pp. 65 *et seqq*.
[5] Cf. Dobell and O'Connor (1921), p. 68, footnote 2. I still find no reason to modify the views there expressed.
[6] See p. 566 *infra*.

Another physiological difference discovered in Strain *T.* is noteworthy. From time to time I tested all my strains to see whether they would ingest human red blood-corpuscles. Strain *T.* was the only one which would never do so—no individual of this strain ever having been seen containing a single ingested red-cell in any experiment. I have as yet found no other strain of *Trichomonas* from men or monkeys showing this peculiarity. All the others here described ate human red blood-corpuscles with avidity whenever they were added to the cultures, and never lost the power of doing so—no matter how long they were cultivated. Strain *T.* was also remarkable because it would hardly ever ingest solid rice-starch[1].

In size and shape all strains showed great variation when grown in different media, and at different stages of incubation. The flagellates are largest in young cultures, and in those containing starch they are often enormous—far larger than any specimens found in the bowel or in faeces. In old cultures, after prolonged incubation, they are generally small and often minute. Moreover, the morphology and the magnitude of trichomonads may both be profoundly modified by the accompanying bacterial flora and the reaction of the medium. It is therefore impossible to compare any one strain exactly with any other unless both can be cultivated in precisely the same way and with identical bacteria. This was never possible with any of the strains of *Trichomonas* used in my experiments, so minor differences between them—almost certainly due to the causes indicated—cannot be discussed with any profit. After prolonged study of all the strains here mentioned—living in various hosts, and growing in divers culture-media and under different conditions—I see no good reason to believe that they belong to more than one single species.

(4) *Methods of inoculation and examination*

In previous instalments of these *Researches* I have described my methods of inoculating animals with amoebae. I used a similar technique in my experiments with trichomonads, so it is only necessary to remind the reader here that all my monkeys were tame and well trained for present purposes. Feeding on cultures of *Trichomonas* was accomplished in every case without the slightest difficulty—the monkey swallowing the entire dose from a pipette put into its mouth. Forcible feeding, or injection of material through a stomach-tube, was never necessary. Every experiment was performed in the most natural way possible.

As in my experiments with amoebae, it has been my practice to test the

[1] The flagellates of Strain *T.* also possessed conspicuously smaller mouths than those of the other strains: and I have sometimes been tempted to believe that it was for this reason that they so seldom ate anything larger than bacteria. But this purely mechanical "explanation" seems unsatisfactory when one observes trichomonads of other strains containing ingested starch-grains so large that they almost fill their bodies, and which appear far too large ever to have entered by the mouth. There can be no doubt that the oral orifice of *Trichomonas* is capable of tremendous stretching, and can admit particles apparently far beyond its capacity when undilated.

viability of all infective material employed. A small quantity of the inoculum —usually the drainings of the pipette or culture-tube, after feeding—was incubated in suitable culture-medium, in order to make sure that the flagellates were really alive and healthy. All such controls invariably gave satisfactory results: and though they may seem superfluous, they often served to ratify a "negative" animal-experiment.

As *Trichomonas* is so insusceptible to cold, I took no precautions to keep the flagellates warm before inoculation. All feedings were conducted at ordinary room-temperature, with cultures taken from the incubator a few minutes previously and not protected from cooling.

Rectal inoculations into monkeys I make through a greased rubber catheter passed into the intestine through the anus to its full length (about 20 cm.). When the tube is properly inserted a syringe charged with the inoculum is attached to the free end, and its contents slowly discharged into the bowel. (Such injections should only be given to a macaque just after it has defaecated spontaneously: otherwise the introduction of the tube and the inoculum causes almost immediate evacuation of the rectal contents, and consequent elimination of most of the material injected.) Vaginal inoculations I made with a glass pipette of suitable size, inserted as far as possible. No force was ever used.

Examinations of intestinal contents for trichomonads were always made upon freshly passed material collected by myself and with all the necessary precautions. I never made cultures from stale faeces; and all the microscopical findings here recorded are therefore founded on faecal specimens examined immediately after their natural discharge from the body.

The vaginal secretions of *Macacus sinicus* are not easy to examine microscopically, so I add a few further notes on my technique in this connexion. Females of this species (*M. sinicus* = *Silenus sinicus* seu *radiatus* = *Macaca radiata*) frequently discharge large quantities of thick, viscid mucus from the vagina[1]; and this secretion is often so copious, so tenacious, and so intractable, that it is practically impossible either to inoculate a monkey satisfactorily *per vaginam* with trichomonads or to examine her vaginal discharges afterwards. The clear, ropy, and elastic mucus can easily be drawn out into threads more than a yard long, but it is extremely difficult to get a separate sample of it between a slide and coverslip or into a culture-tube. I noticed, however, that the vaginal discharges are always thickest and stickiest midway between one menstruation and the next: before, during, and just after menstruation the secretion is comparatively thin and easy to deal with.

Accordingly, in the one experiment here recorded (with Susanna[2]) in which I inoculated *Trichomonas* intravaginally, I made the injection a few days before

[1] According to Zuckerman (1932, p. 89), the secretion really comes from the *cervix uteri*. My own observations are based chiefly upon a study of my monkey Susanna, but Dr Zuckerman informs me that the phenomena noted are characteristic of all females of this species which he has observed in the London Zoo.

[2] See p. 556 *infra*.

menstruation was due[1]. I thus had no difficulty in introducing the inoculum, and made certain that it was retained in its entirety.

After trying various methods of examining the vaginal contents of macaques culturally, I have found the following to be the best: A small sterile cotton-wool swab tied on the end of a thin wooden stick is passed into the vagina, and gently agitated so as to absorb and collect as much as possible of its contents. The swab is then thoroughly rubbed up in a test-tube containing 5 c.c. of dilute horse-serum (1 : 8 parts of Ringer's fluid): and the resulting suspension is then poured on to a slope of inspissated horse-serum, and incubated as an ordinary solid-liquid culture. In making such cultures I always take every aseptic precaution but disregard the temperature.

When I make intrarectal or intravaginal inoculations, or take vaginal swabs of any monkey, I carry out all the necessary manipulations single-handed. The only assistance I receive is from the monkey itself—all my animals having been taught to submit to any painless experiments I desire to perform. For a vaginal or rectal injection the monkey stands with its head and shoulders down, its tail and hindquarters raised and presented to me[2]. I grip the tail near its root in my left hand—to keep it out of the way, and steady the animal —and make the necessary injection or swab with my right hand. The employment of violence, or even roughness, is as unnecessary as it is undesirable. A willing macaque is more serviceable as a collaborator than a powerful laboratory assistant, and the time spent in training is well repaid by the ease and certainty with which all such operations can be performed afterwards. My monkey Susanna, for example, will—for me—remain stationary in any position for any length of time, though she will not let anyone else even touch her. She will allow me to insert a tube into her colon, and will then remain motionless—though unrestrained in any way—while I walk about the room, charge the syringe, and carry out the inoculation at my leisure.

I may add here, in conclusion, that "negative examinations"—when made with all proper precautions, and confirmed by careful study of cultures inoculated with suitable samples—have a much higher value, as evidence of non-infection, for *Trichomonas* than for any other intestinal protozoon of men or macaques. In my experience, it is almost impossible not to discover an infection by proper cultural examination of any infected animal. *Trichomonas*, when present even in numbers microscopically undiscoverable in the faeces, can almost invariably be quickly detected by appropriate methods of cultivation. It is recoverable so readily, indeed, as to constitute a real nuisance in the study of concomitant protozoa: and in my own case, accordingly, by infecting myself with this flagellate experimentally I have not only increased the difficulty of isolating other protozoa from my stools enormously, but have almost ruined myself as a subject for future researches.

[1] I knew the approximate date, because I kept a record of Susanna's menstrual periods for several years before and after the experiment.

[2] It is easy to teach macaques to adopt this attitude, as it is the posture naturally assumed when they wish to show friendliness or submission to other monkeys.

III. EXPERIMENTS AND RESULTS

In this section I shall record, in some detail, all the attempts which I have hitherto made to implant various strains of *Trichomonas* in divers primate hosts. The strains employed have just been briefly described, and the essential data regarding the experimental animals and general methods have been duly noted. If the reader will bear in mind what I have already said, therefore, I think he will easily be able to understand the records which now follow—without further preamble.

I must note here, however, that all the following experiments have been made in conjunction with others which are as yet only partially recorded. Much of the work now summarized has run concurrently or intercurrently with other researches which are still unfinished or unpublished. Moreover, I have condensed and simplified my actual findings considerably, in order to present my results as clearly as possible. I am fully alive to the importance of publishing exact data in studies of the present sort, but have purposely eliminated masses of carefully recorded detail whenever such *minutiae* appear to me inessential. For example, I generally give the exact dates on which experiments were performed, and the precise numbers of positive or negative examinations made before and after—since without such information the reader cannot fairly assess the value of the observations: but I do not usually record here the actual dates on which negative or positive examinations were made over a long period, if such dates seem to me immaterial[1].

(1) *Attempt to infect* M. rhesus *with a* Trichomonas *of* M. rhesus

I describe this experiment first—though it was not the first I made—because it is obviously of prime importance to know the results of the simplest and most "natural" experiment before we consider attempts at cross-infection. In the present case, the result was unexpected; but it taught me once more that in work of this sort nothing should be taken for granted.

The experiment consisted in feeding an uninfected *M. rhesus* on a "pure" culture of *Trichomonas* derived from another monkey of the same species, in order to ascertain whether infection could be induced by such means. I imagine that everybody, at the time of the experiment, would have regarded a positive outcome as inevitable.

The monkey used was **Jacko**, a healthy young male *M. rhesus*, and the experiment was performed in August 1925. Before this date, I had examined Jacko's faeces exhaustively for over a year (from 7. vii. 24 to 19. viii. 25), both microscopically and culturally, and had made numerous experiments with his intestinal protozoa. In more than 33 examinations made during this period no *Trichomonas* was ever found in him, and I am therefore confident that he was naturally uninfected with this flagellate.

[1] Additional details from my notebooks will be gladly supplied to any fellow-worker on demand.

On 28. viii. 25 Jacko swallowed all the liquid and sediment in a rich 3-day culture of Strain *T.*[1] (30th serial subculture, in "HSre" medium), containing many thousands of active and healthy trichomonads. During the next few weeks (until 30. ix. 25) I examined his faeces frequently, with great care (24 times microscopically, 8 times culturally—using various media), but no *Trichomonas* was ever discoverable. Thereafter I studied his faeces in great detail, in the course of other experiments, and they remained consistently negative to the day of his death (15. ii. 28). Altogether, I have recorded 75 negative microscopic examinations since the experimental feeding, and 51 negative attempts to recover trichomonads from his excreta by cultural methods.

I think the evidence is here conclusive. For more than a year Jacko was uninfected with *Trichomonas*: he was then fed on a pure culture of this flagellate, derived from another monkey of the same species: and for nearly $2\frac{1}{2}$ years thereafter he remained uninfected. This was a clean and carefully controlled experiment, and its result appears to me definite and unequivocal.

(2) *Attempts to infect* M. sinicus *with a* Trichomonas *of* M. rhesus

Although the foregoing experiment ultimately proved negative, I was not aware of this at the time, and attempted almost simultaneously to infect 2 individuals of a different species (Mungo and Susanna, *M. sinicus*) with the *Trichomonas* (Strain *T.*) which I had isolated in culture from *M. rhesus*. These experiments must now be noted.

(*a*) **Mungo**. This young male *M. sinicus* was studied for 4 years—from 7. viii. 24 to 8. viii. 28, when he was killed. During the first year that he was in my possession I examined his faeces very thoroughly on 24 recorded occasions, and investigated his natural intestinal infections with flagellates and amoebae in great detail. By 27. vii. 25 I was satisfied that he was uninfected with *Trichomonas*, but I made a further examination on this date with the usual negative result. (No culture was inoculated on this occasion, but a negative culture was made on 21. vii. 25.) I therefore regarded Mungo as a "clean" animal, and attempted to infect him about a fortnight later.

On 13. viii. 25 I fed Mungo on all the liquid and sediment in a rich 2-day culture of *Trichomonas* (Strain *T.*, 29th serial subculture in "Ehs" medium). After this date I examined his faeces very carefully—both microscopically and culturally—every day for a week (14. viii. 25 to 20. viii. 25) with consistently negative results. No infection was demonstrable, so I decided to try again.

On 22. viii. 25 therefore I fed Mungo on another rich culture of *Trichomonas* (Strain *T.*, 29th serial subculture in "HSre" medium, 2 days old), and examined his faeces afterwards for 12 days. All examinations were once more completely negative (6 examinations, both microscopic and cultural, 23. viii. 25 to 4. ix. 25).

[1] For details of this strain, and all others used in subsequent experiments, the reader is referred to Table I, p. 540 *supra*.

As the two foregoing experiments—contrary to my expectations—appeared to have failed completely, I thought it possible that my technique was to blame. I had been careful to administer very fresh cultures, still warm from the incubator (so as not to injure the trichomonads in any way), but it occurred to me that Nature probably took no such precautions. Possibly the flagellates needed cooling before they became infective: so I made a further experiment to test this point.

On 4. ix. 25 I fed Mungo on another rich culture of *Trichomonas* (Strain *T.*, 32nd serial subculture, in "HShs" medium), which I had kept previously for a day at room-temperature after removing it from the incubator[1]. The flagellates were then still active and healthy in appearance, despite their cooling. I examined Mungo's faeces very carefully for a further week, but all findings were again quite negative (8 consecutive examinations, made both microscopically and culturally).

After this I used Mungo for other experiments; but as he was apparently still uninfected with *Trichomonas* six months later, I made a final attempt to infect him with the same strain as before. On 2. iii. 26 I fed him on the contents of 2 very rich and fresh 2-day cultures (Strain *T.*, 63rd serial subcultures in "Ehs" and "HSre" medium), and re-examined his faeces with the utmost care for the next fortnight—by direct microscopic examination and by culture (and subculture when necessary). All the findings were once more completely negative.

This monkey was used later for experiments with *E. histolytica*, and he was twice treated with emetine in 1927 (6. i. 27–29. i. 27 and 20. ii. 27–8. iv. 27). During these treatments, and afterwards, his faeces were examined exhaustively till the day of his death (8. viii. 28); but *Trichomonas* was never found. Altogether, he was examined 105 times microscopically and 99 times culturally after the last attempt to infect him in 1926. I feel confident, therefore, that he was neither infected with *Trichomonas* naturally, nor experimentally infected by me in any of my 4 attempts. At all events, 4 separate feedings on very rich cultures ought to have caused infection if he were infectible: and more than 100 negative microscopic and cultural examinations (made over a period of $2\frac{1}{2}$ years after the last feeding) furnish strong evidence that this macaque was incapable of being infected with Strain *T.*

(*b*) **Susanna.** This female *M. sinicus* has now been in my possession for 10 years, and has been studied continuously and experimented upon very frequently. In the present experiments she was used in a further attempt to infect a macaque of her species with *Trichomonas* from *M. rhesus*. As experiments with flagellates administered *per os* had all failed, up to this time, I tried to infect Susanna with the same strain by injecting cultures *per anum*.

Before this experiment Susanna's faeces had been examined exhaustively for over a year, and all her intestinal protozoa had been studied in great detail during this period. Between 24. vii. 24 and 4. viii. 25 I have recorded 57 nega-

[1] Actually for $21\frac{1}{2}$ hours at a temperature of approximately 15° C.

tive microscopic examinations for *Trichomonas*, and 9 exhaustive negative attempts to recover this organism by cultural methods: and I am satisfied that she was uninfected at the date of the experiment.

On 13. viii. 25 I inoculated Susanna intrarectally with all the liquid and sediment in a rich 2-day culture of *Trichomonas*, Strain *T.* (29th serial subculture in "Ehs" medium). During the next 8 days I examined her faeces very carefully, both culturally and microscopically, every day; but all examinations were entirely negative.

On 22. viii. 25 I gave Susanna a second intrarectal inoculation with Strain *T.* (another 2-day culture, 29th cultural generation, in "Ehs" medium). This was less satisfactory than the first, as she passed a small amount of faeces *per anum* about 5 minutes after the injection. The inoculum was very rich in trichomonads, however, and part of it at least must have been retained. Six very careful microscopic and cultural examinations made during the next four weeks were all completely negative for *Trichomonas*.

After this experiment I continued to study Susanna, and used her for other experiments. I did not attempt to infect her with *Trichomonas* again, however, for over 3 years. The attempt then made will be described later[1]: and I will only add here that from 22. viii. 25 to 26. ix. 28 I made and recorded 23 negative microscopic and 16 negative cultural examinations of her faeces; and I satisfied myself thereby that she was not infected with *Trichomonas* as a result of my intrarectal administrations of Strain *T.*

The two foregoing series of experiments with Mungo and Susanna showed that the *Trichomonas* (Strain *T.*) which I had cultivated from *M. rhesus* was not transmissible to two particular individuals of *M. sinicus*—whether introduced *per os* or *per anum*. They obviously do not warrant the assumption that all individuals of *M. sinicus* are incapable of infection with all strains of *Trichomonas* from *M. rhesus*.

(3) *Attempt to infect* M. rhesus *with a* Trichomonas *of* M. nemestrinus

All the experiments hitherto recorded were failures. I failed completely to infect a *Macacus rhesus* with a *Trichomonas* obtained from the same species of host, and also to infect two *M. sinicus* with the same flagellate. But I now have to record a partial success in a similar attempt at cross-infection.

The monkey used for this experiment was **Rosa** (*M. rhesus*), and the trichomonads used were of Strain *NT.*—derived from *M. nemestrinus*. The experiment was performed in 1928, and the essential data are as follows:

Rosa was under observation, and used for various experiments[2], from October 1926 until June 1927—approximately 8 months. During this period she was very carefully studied by Miss A. Bishop—under my supervision—and found to be negative for *Trichomonas*. She came into my possession on

[1] See p. 550 *infra*.

[2] By Dr J. A. Campbell. Cf. Bishop (1927), Dobell with Bishop (1929), Dobell (1931).

25. vi. 27, in a very weak state: and I kept and studied her for about 15 months before I made the present experiment. She was then in excellent condition, very healthy and growing actively, and had completely recovered from the effects of her earlier experiences.

Between 4. ii. 27 and the beginning of September 1928 Rosa's faeces were examined microscopically by Miss Bishop on 78 occasions; and cultures, in various media, were made and studied by her 73 times, on various dates. Before the present experiment I also examined her carefully, both microscopically and culturally, several times. *Trichomonas* was never discoverable in her dejecta, and she was never in contact with any other monkey infected with this flagellate during this period. The evidence that Rosa was not naturally infected with *Trichomonas* is therefore very strong.

On 26. ix. 28 I fed Rosa on all the trichomonads in two rich 2-day cultures of Strain *NT.* (132nd serial subculture, in "Ehs" medium). In this experiment the flagellates were administered with a little milk[1], to make the material more palatable. It was all swallowed readily; and afterwards the monkey's faeces were carefully examined daily—both microscopically and culturally—by Miss Bishop or myself. They remained completely negative for *Trichomonas* for 12 consecutive days (26. ix. 28 to 7. x. 28): and as I then thought the experiment had failed—like those previously performed—I treated Rosa with emetine for 7 days (7. x. 28 to 13. x. 28 inclusive), in order to eradicate her natural infection with *E. histolytica*[2].

The daily microscopic and cultural examinations were continued during and after treatment; and to my surprise Miss Bishop discovered *Trichomonas* in the culture made on October 15—two days after the cessation of the emetine-treatment, and 19 days after inoculation with the flagellates. On this date (15. x. 28) the direct microscopic faecal examination was negative; but for the next 12 days (till 27. x. 28) *Trichomonas* was recovered in every culture made from Rosa's faeces. On 20. x. 28 I isolated a pure strain (*RT.*) which I kept and studied for a long time[3], and which proved to be indistinguishable from its parent (*NT.*). But singularly enough, during the whole of this period when *Trichomonas* was so readily recoverable by cultural methods, it was never possible to discover this organism by direct microscopic examination. Miss Bishop and I searched for the flagellates very carefully in Rosa's faeces at this time, but always without success.

On 28. x. 28 not only were the microscopic examinations negative but the cultures were too: and after this date *Trichomonas* was never discoverable in Rosa by any method. The monkey remained negative until 2 April 1930, when I made another attempt to infect her[4]. During this period—over 15 months—I examined her faeces 208 times microscopically and 204 times culturally, on

[1] I had previously determined by experiment that *T. hominis* (and similar flagellates from macaques) can live in milk for many hours at least.

[2] See Dobell with Bishop (1929).

[3] Cf. p. 540 *supra*. Further experiments with this strain are described in the present paper.

[4] Cf. p. 551 *infra*.

various dates, and always with entirely negative results[1]. It is inconceivable that an infection with *Trichomonas* could remain undiscovered by such prolonged and exhaustive examination.

The result of this experiment appears, therefore, to be quite definite. It showed that a strain of *Trichomonas* derived from *M. nemestrinus* was able to infect an individual of *M. rhesus* for a period of at least one month[2]; and that the infection then died out. Although the subject of the experiment was treated with emetine afterwards, it is most unlikely that this influenced the result in any way. Indeed, the fact that *Trichomonas* was first recovered from Rosa's faeces in cultures made 2 days after her first course of treatment, confirms the conclusion that this alkaloid has no specific curative action on simian infections with trichomonads[3].

(4) *Attempts to infect* M. sinicus *and* M. rhesus *with the* Trichomonas *of Man*

I have made two attempts to transmit *Trichomonas hominis*—the ordinary intestinal trichomonad of Man—to macaques (*M. sinicus* and *M. rhesus*). Both experiments were made with the same strain of flagellate (*FT.*), and the monkeys used were again Susanna and Rosa. These attempts must now be recorded.

(*a*) **Susanna** (*M. sinicus*). I have already described[4] how I tried unsuccessfully to infect this macaque by intrarectal injection of cultures (Strain *T.*, from *M. rhesus*); and at the date of the present experiment—made in 1928—no trichomonads had ever been found in her faeces by microscopic examination, or in cultures made therefrom by various appropriate methods, for over 4 years (including the periods of previous experiments). Altogether about 90 negative microscopic examinations had been made, and about 40 equally negative cultural attempts to demonstrate *Trichomonas* in her excreta. I was therefore fully satisfied that she was uninfected.

On 26. ix. 28—over 3 years after the last attempt with Strain *T.*—I fed Susanna on all the trichomonads in two rich 2-day cultures of Strain *FT.* (234th serial subcultures, in "HSre" medium), mixed with a little milk. Her faeces were then examined[5] every day—both culturally and microscopically—for the next 11 days with completely negative results.

Susanna was then treated with emetine, to remove her natural infection with *E. histolytica*. Afterwards she was reinfected with this species of amoeba, and twice re-treated with emetine and reinfected with other strains of the

[1] This monkey was treated with emetine twice during this period, and experimentally infected and reinfected with *E. histolytica*. See Dobell with Bishop (1929) and Dobell (1931) for additional particulars.

[2] Though *Trichomonas* was demonstrable only from 15. x. 28 until 27. x. 28, the infection must have been present—though at first undetected—from 26. ix. 28 (the date of feeding) until the last date on which it was discovered (27. x. 28).

[3] Cf. p. 537 *supra*.

[4] Cf. p. 547 *supra*.

[5] Partly by Miss Bishop.

same parasite[1]. In the course of these experiments her faeces were examined in great detail until April 1930. During this period of approximately 1½ years I have recorded the findings from 235 microscopic and 205 cultural examinations—all completely negative for *Trichomonas*.

This evidence appears to me sufficient to prove that Susanna (*M. sinicus*) remained uninfected after swallowing a vast number of fresh and active trichomonads (*T. hominis*) directly derived from Man. After this unsuccessful attempt to infect her, I used Susanna for some further experiments, which will be described in later sections[2].

(*b*) **Rosa** (*M. rhesus*). On 2. iv. 30 I made an attempt to infect this monkey with *T. hominis* (Strain *FT.*). As already noted[3], she had then been negative for *Trichomonas*—after my previous experiment with Strain *NT.*—for over 15 months, during which period I had carefully examined her faeces over 200 times. I cannot doubt that she was uninfected at the date of the present attempt.

On this day (2. iv. 30) I fed Rosa on all the sediment from an extremely rich 2-day culture of *T. hominis* (Strain *FT.*, 373rd serial subculture, in "HSre" medium), mixed with a little pasteurized milk. The material was very fresh—having been administered about 5 minutes after the culture was removed from the incubator. In order to be quite certain that neither the slight cooling—which inevitably occurred in performing the experiment at room-temperature—nor the admixture with milk had killed or injured the trichomonads, I kept the test-tube in which the inoculum was prepared for a further 2½ hours on my laboratory bench, and then inoculated the scanty drainings into a tube of culture-medium and put it in the incubator. It yielded a good growth of normal *Trichomonas* on incubation.

After this experimental feeding I examined Rosa's faeces daily for the next 38 days, both microscopically and culturally every time (3. iv. 30 to 10. v. 30). All the examinations were entirely negative for *Trichomonas*, so I was satisfied that no infection had resulted.

Since this date (10. v. 30) I have used Rosa for various other experiments, in the course of which I have examined her faeces very thoroughly. Up to the time of writing (June, 1934) I have made altogether 148 further microscopic examinations and 147 additional cultural tests on various dates during the last 4 years. They have all been consistently negative.

It is thus clear that Rosa (*M. rhesus*) remained uninfected after ingesting a very large number of fresh and active *T. hominis* (Strain *FT.*). If the results of this experiment be added to those previously recorded with Susanna, they show pretty conclusively that these two macaques (*M. rhesus* and *M. sinicus*) were not infectible with Strain *FT.* (*Trichomonas hominis*) administered *per os*. The experiments were not repeated—through lack of time and opportunity—

[1] Cf. Dobell with Bishop (1929) and Dobell (1931).
[2] Cf. pp. 554 and 556 *infra*.
[3] Cf. p. 549 *supra*.

but I see no reason to doubt the obvious conclusion to be drawn from these two very carefully controlled series of observations.

I may add here that none of the animals used in any of these experiments ever contracted dysentery or diarrhoea or any other illness as a result of my inoculations.

(5) *Attempts to infect Man with Trichomonads from Macaques*

For the purpose of the present researches it has been necessary not only to endeavour to infect macaques with the trichomonads of Man, but also to ascertain whether human beings can acquire infection with trichomonads from such monkeys. So far, I have been able to make only two experiments in this connexion—chiefly for want of suitable human subjects. Both experiments were made upon myself.

I have already given some account of my intestinal protozoal fauna in a previous instalment[1]. Assuming that the reader has read what is there recorded, I shall now add merely a few particulars of immediate importance. My object now is to show that I was uninfected with *Trichomonas* at the time when I first attempted to infect myself with a simian species of this genus. I made this attempt in August 1925, and to the best of my knowledge and belief was then a "clean" subject for experiment. The evidence for my non-infection is as follows:

From the end of 1906 until August 1925 (nearly 19 years) I had examined my stools microscopically—at various dates, but chiefly during the last ten years—over 200 times. (Actually I have recorded the results of only 181 examinations made during this period, because I often omitted to make a note of examinations which were entirely negative for all protozoa.) All these examinations failed entirely to reveal the presence of any infection with *Trichomonas*. Although they were, alone, sufficient to convince me that I did not harbour this flagellate, I made 7 additional attempts—shortly before the present experiment—to discover trichomonads in my stools by appropriate cultural methods. All these efforts were likewise completely unsuccessful. *Trichomonas* was never demonstrable in me, after exhaustive study, during some 19 years previous to 31. viii. 25.

(*a*) *Experiment with Strain* T. On 31. viii. 25 I swallowed all the trichomonads in a very rich 3-day culture of Strain *T.* (31st serial subculture in "HSre" medium), derived from *M. rhesus*. The entire sediment in the culture-tube—consisting of *Trichomonas* and unidentified bacteria—was thickened with a little sterile rice-starch, and introduced into 3 small gelatin capsules, which I swallowed at once with a draught of water on an empty stomach. I experienced no unpleasant consequence of any sort—remaining normal in every way after the experiment.

[1] Cf. Dobell (1933), p. 450 *et seq.*

I continued the daily examinations of my stools afterwards, both microscopically and culturally, for the next 18 days, but always with entirely negative results (1. ix. 25 to 18. ix. 25). As it appeared probable that the attempt had failed, I then continued the examinations at longer intervals—very irregularly, and usually in association with later experiments—until I was satisfied of the result. Altogether, I examined my stools on various dates during the next 4⅓ years (September 1925 to January 1930) 130 times microscopically and 118 times by carefully controlled cultures. All these examinations were completely negative for *Trichomonas*: and I am therefore confident that I acquired no infection as a result of ingesting many thousands of active and fresh trichomonads (Strain *T.*, from *M. rhesus*) in 1925. The evidence appears to me conclusive.

As I was satisfied by January 1930 that I was still uninfected with *Trichomonas*, I regarded myself as still a suitable subject for experimentation and made a further attempt to infect myself by means of another simian strain.

(*b*) *Experiment with Strain* RT. The trichomonads used for this attempt belonged to a strain whose origin and history have already been briefly noted[1]. It was derived from *Macacus nemestrinus* (3. viii. 27), cultivated *in vitro* for nearly 14 months (until 26. ix. 28), and then inoculated into Rosa (*M. rhesus*) in whom it produced a temporary infection (lasting about a month). During the time of her infection (on 20. x. 28) I isolated Strain *RT.* from her faeces, and kept it in cultures for over 16 months (until 3. iii. 30, when it was voluntarily abandoned). Strain *RT.* was therefore a thoroughly studied simian strain of *Trichomonas* originating in *M. nemestrinus* but passed through *M. rhesus*.

On 22. i. 30 I swallowed all the trichomonads in a rich 2-day culture (Strain *RT.*, 70th serial subculture, in "Ehs" medium). In this experiment I mixed all the sediment (*Trichomonas* + unknown bacteria) in the culture-tube with 10 c.c. of sterilized milk, and drank it at once on an empty stomach. My stools had then been negative for *Trichomonas* during at least 23 years, and for more than 4 years (130 examinations) since the previous experiment.

After swallowing the trichomonads on 22. i. 30 I continued to examine my stools daily—both microscopically and culturally—for 22 days (23. i. 30 to 13. ii. 30). On the 6th day after the experiment (28. i. 30) the culture gave a positive result for the first time—numerous trichomonads developing on incubation: and afterwards positive cultures were almost always obtained. No trichomonads were discoverable by direct microscopic examination, however, until the 13th day (4. ii. 30), when I found a very few flagellates after prolonged search.

Since this date (4. ii. 30) I have continued to examine myself intermittently until the time of writing (June, 1934). During this period I have made 130 microscopic examinations of my stools on various days, and have invariably made and investigated cultures simultaneously. Of the direct

[1] Cf. pp. 540 and 549 *supra*.

examinations 51 have been positive for *Trichomonas*, and 79 have been microscopically negative. Cultures have yielded positive results 128 times.

Although the flagellates have always been easily demonstrable in me, by cultural methods, ever since the end of January, 1930, until the present time (June, 1934—approximately $4\frac{1}{2}$ years), they are usually difficult to discover by direct microscopic examination of my stools, and have never been found in them plentifully[1]. I infer, from all the findings, that my infection is not heavy; but it otherwise differs in no way from any natural human infections which I have studied in numerous human beings. Morphologically, all the strains which I have recovered at various times from myself are typical *T. hominis*, and indistinguishable from their simian parent-strains (*NT.* and *RT.*). One of the strains which I isolated in "pure" culture was studied in particular detail; and with this strain (*DT.*) I made an attempt to infect *M. sinicus*. This will be described immediately (see the following section).

I need only add here that this experiment has convinced me personally that I have succeeded in establishing in myself—more or less permanently—a strain of *Trichomonas* (indistinguishable in any way from typical *T. hominis*) which was derived originally from monkeys of the genus *Macacus* [=*Macaca*=*Silenus*]: and that, as I anticipated, I have suffered no noticeable harm from my experimental infection.

(6) *Attempt to infect* M. sinicus *with a* Trichomonas *of* M. nemestrinus *after its passage through* M. rhesus *and Man*

This is the last experiment which I made in my efforts to establish the trichomonads of one primate in the intestine of another. It was made with my monkey **Susanna** (*M. sinicus*) and *Trichomonas* Strain *DT.*, which—as just recorded above—was derived from my own stools after I had succeeded in infecting myself experimentally with a *Trichomonas* of *M. nemestrinus* passed through *M. rhesus*.

At the time of the attempt (April, 1930) Susanna's faeces had been consistently negative for *Trichomonas* during a period of about $5\frac{3}{4}$ years. From 24. vii. 24 until 3. iv. 30 she was under continuous observation and used for repeated experiments, including 3 unsuccessful attempts to infect her with the trichomonads of *M. rhesus* and of Man[2]. Since the last attempt (26. ix. 28) her faeces had been examined 235 times microscopically and 205 times by means of cultures: and during the whole period of previous investigation she had been carefully studied altogether 323 times by direct microscopic examination, and 238 times culturally[3]—always with negative results so far as *Trichomonas* is concerned. Moreover, Susanna had—to my certain knowledge

[1] Many of the "positive" records are based upon the finding of only 1 or 2 trichomonads in the preparation examined microscopically.

[2] Cf. pp. 547 and 550 *supra*.

[3] These figures represent the examinations entered in my records. Susanna was actually examined more frequently by me, and was also studied by Miss Bishop—of whose examinations I kept no notes, as they merely confirmed my findings.

—never at any time been in contact with any monkey infected with this flagellate; so I am confident that she was uninfected in April 1930, when I performed the present experiment.

On 3. iv. 30 I fed Susanna on a rich 3-day culture of Strain *DT*. (14th serial subculture, in "HSre" medium). The sediment in this culture was mixed with 5 c.c. of pasteurized milk before administration: and the scanty remains of the mixture after ingestion, inoculated into suitable medium and incubated, gave a copious growth of *Trichomonas*—proving that the inoculum was viable.

After inoculation I continued to examine Susanna's faeces daily—microscopically and culturally—until 10. v. 30 (=37 consecutive days). The results were somewhat surprising. All microscopic examinations were completely negative for *Trichomonas* until April 25 (=22 days), when I found a few individuals in her faeces for the first time. All the cultures were, with a single exception, negative until April 20 (=17 days), when trichomonads developed fairly plentifully on incubation: and after this date they were recovered regularly. The exception just noted occurred on April 5 (=2 days after inoculation). On this date both microscopic examination and primary culture were negative for *Trichomonas*: but in a subculture made from the primary culture, I obtained later a good growth of the flagellates[1]. After this date I was never able to recover them again in cultures or subcultures until 20. iv. 30.

From April 25 (when trichomonads were first discoverable both by direct examination of the faeces and in cultures made therefrom) until May 10 (when the daily examinations were discontinued) the infection ran a normal course. During this period of 16 days, the faeces were positive for *Trichomonas* 13 times, and negative on only 3 days; while the cultures gave a positive result every day. I was therefore satisfied that the monkey had acquired an infection, and examined her faeces intermittently afterwards in order to ascertain whether or no it would persist. I imagined that it would, as trichomonads were often observable plentifully and always developed abundantly in cultures.

In the middle of May, however, I made two examinations (13. v. 30 and 20. v. 30) in both of which no *Trichomonas* was discoverable microscopically in the stools though the cultures from them were both positive: and after the last date I never succeeded in finding any trichomonads in Susanna's faeces by any method of examination. In spite of all my efforts, she remained obstinately negative for about 8 months. During this period (May 1930 to January 1931) I examined her faeces very carefully—microscopically and culturally every time—on 42 different days. As I had then fully satisfied myself that she could not possibly harbour an intestinal infection with *Trichomonas*, I used her for another experiment which will be described in the next section.

The final result of this attempt, therefore, showed that Susanna (*M. sinicus*) acquired an infection with Strain *DT*. (*Trichomonas* of *M. nemestrinus*, after passage through *M. rhesus* and Man): and that this infection, which appeared

[1] These findings indicate that the sample of faeces inoculated into the culture-tube on 5. iv. 30 probably contained only a single trichomonad.

at first to be solid and permanent, endured nevertheless for only 1½ months (47 days—3. iv. 30 to 20. v. 30) and then died out spontaneously.

(7) *Attempt to establish an intestinal strain of* Trichomonas *in the vagina of a macaque*

This is the last infection-experiment (to the date of writing) which I have performed with the trichomonads of the Macaques and Man. I am aware that it may appear, to other workers, rather rash after all my previous experiences, which at first sight seem so inconclusive and conflicting. But I had good reasons for making it, and its results have justified my temerity.

As everybody now knows, human beings are sometimes inhabited by *Trichomonas* not only in their intestines but also in their genito-urinary apparatus[1]. The human vagina, and the male urethra (and bladder?), occasionally harbour trichomonads ("*T. vaginalis*") which are not certainly distinguishable specifically from *T. hominis* (of the bowel). Some workers believe that "*T. vaginalis*" and "*T. hominis*" are identical organisms living in different sites in the human body; but many more regard them as separate species. At the present moment, however, no conclusive evidence has been adduced to prove whether they are specifically different or identical. As I have long been interested in this problem I made the present experiment. I tried to find out whether an intestinal *Trichomonas*—able to live in the intestines of several monkeys and a man—could establish itself in the vagina of a macaque.

The macaque used for the attempt was **Susanna** (*M. sinicus*), already mentioned in connexion with many earlier experiments. She was an original member of my small family of tame experimental monkeys, and has never been in contact with any animal infected with *Trichomonas* since she came into the Institute in May, 1924. All my monkeys were naturally uninfected with trichomonads of any sort, in any part of the body (mouth, intestine, vagina, etc.), so far as I could ascertain by careful examination conducted over long periods. It will be recalled, however, that I made four attempts to infect Susanna—as noted earlier in the present paper[2]—by administering large doses of these flagellates derived from various extraneous sources. The first two attempts (with Strain *T.*, from *M. rhesus*; *per anum*, 13. viii. 25 and 22. viii. 25) were entirely unsuccessful, as was also the third (with Strain *FT.*, from Man; *per os*, 26. ix. 28): but at the fourth attempt (with Strain *DT.*, derived from *M. nemestrinus* but afterwards passed through *M. rhesus* and Man; *per os*, 3. iv. 30) I succeeded in infecting her, intraintestinally, for a period of over 6 weeks. After this she remained consistently negative for *Trichomonas* for nearly 8 months (May 1930 to January 1931), during which time I examined her faeces—both microscopically and culturally on every occasion—very carefully on 42 several days. By the end of January (1931) I was satisfied that

[1] I leave out of account the trichomonads of the mouth ("*T. buccalis*"). These are probably of a distinct species, about which I hope to say more on a future occasion.

[2] Cf. pp. 547, 550, 554 *supra*.

Susanna's intestinal infection had died out, and I therefore regarded her as a "clean" animal suitable for further experiments with trichomonads.

I was not only convinced, at the date of the present experiment, that Susanna harboured no trichomonads in her intestines, but also that she had no vaginal infection: because I had examined her vaginal secretions microscopically on many occasions since she first came into my possession, and had also made many cultures from them—always with negative results. But I usually made no note of such examinations, as they were done casually in the hope of discovering any protozoa which might be present, and not with the specific aim of proving the absence of *Trichomonas* for the purpose of the present experiment. Before performing this, therefore, I subjected Susanna to further tests with the special object of demonstrating the presence or absence of trichomonads in her vagina.

For 7 consecutive days—15–21. ii. 31—I made a most careful study of Susanna's vaginal contents. Each day I collected the largest possible amount of secretion on a sterile swab; I examined a sample of it microscopically; and I then inoculated all the rest into culture-medium particularly favourable to the growth of *Trichomonas*[1]. After incubating these cultures for various periods—and, when it seemed advisable, making and examining subcultures—I proved to my own satisfaction that no trichomonads (or any other protozoa) were present in Susanna's vagina. All the examinations were completely negative.

At this time, and throughout this experiment, Susanna was frequently in the company of another female macaque (Rosa, *M. rhesus*). These were the only monkeys I then had: and though they were caged separately at nights and feeding times, they were allowed to associate freely when out-of-doors during the daytime. To exclude the possibility of accidental infection, therefore, I subjected the Rhesus monkey (Rosa) to similar examination. She was—as she still is—negative for *Trichomonas* in every situation. No trichomonads were discoverable in her vaginal secretions by direct microscopic or cultural examination, and I am convinced that she was uninfected[2].

On 21. ii. 31 I inoculated Susanna intravaginally with a very rich 24-hour culture of *Trichomonas*. The strain used for this experiment was called $\Delta T.$, and was isolated on 7. ii. 31 from my own stools[3] after I had succeeded in infecting myself (on 22. i. 30—more than a year earlier) with Strain *RT*. It was therefore a strain similar to *DT*., with which I had temporarily induced an intestinal infection in Susanna in the previous year, and was likewise ultimately derived from the gut of *M. nemestrinus*. The earlier history of the strain had already proved that these trichomonads were able to produce more or less permanent intestinal infections in a macaque (*M. nemestrinus*) and a man

[1] Cf. p. 544 *supra*.

[2] Rosa—as I may remind the reader—had been previously infected experimentally with Strain *NT*. Cf. p. 548 *supra*. This intestinal infection had died out, however, and her faeces had been negative (as they still are in 1934) since 28. x. 28—*i.e.*, for 2¼ years before the present experiment.

[3] Cf. pp. 540 and 554 *supra*.

(myself), and transient infections in two other macaques (*M. rhesus* and *M. sinicus*).

The subculture of Strain ΔT. used for injection was of the 8th serial generation (in "HShs" medium), and contained thousands of active and healthy trichomonads unaccompanied by any other protozoa. I injected about 2 c.c. of the sediment from this culture, and inserted the pipette so far that its tip was touching the external *os uteri*. I purposely used a small and highly concentrated inoculum and deep inoculation, in order to avoid as far as possible any subsequent expulsion of the material. The operation was completely successful—Susanna remaining quiet throughout, and retaining the entire dose until she went to sleep for the night.

After inoculation I made daily examinations of Susanna's vaginal contents —microscopically and culturally on every occasion—from 22. ii. to 28. iii. 31 (=35 consecutive days). The direct microscopical examinations were negative until 25. ii. 31 (=4 days), when I first found a very few trichomonads in the vaginal mucus. For the rest of this period of daily study (31 days) the microscopical examinations were 11 times positive and 20 times negative for *Trichomonas*. The cultures, however, were with 3 exceptions (1, 14, and 17. iii. 31) positive every day—from the first day after inoculation until the end of the 5 weeks during which the examinations were continued (altogether 32 positives in 35 days). Often the cultures gave very rich growths of typical flagellates, even when the direct microscopic examinations had failed to disclose any after prolonged search.

As I was thus satisfied that Susanna had acquired a vaginal infection I examined her afterwards less frequently—merely making cultures every now and then, in order to determine how long the infection would persist. It has persisted unchanged up to the date of writing—that is, for about $3\frac{1}{4}$ years. Since 28. iii. 31 (5 weeks after infection) I have studied Susanna's vaginal secretions—always both microscopically and by cultures—on 29 different days. Of the direct examinations, 10 have been positive for *Trichomonas*, and 19 negative: while of the 29 cultures, only 7 have been negative[1]—good growths of flagellates being obtained on 22 occasions. Her vaginal infection is still readily demonstrable (June, 1934).

Susanna's vaginal trichomonads can usually be recovered with ease in cultures, but they are always troublesome to find by direct microscopic examination of the secretion. In this they are never present in large numbers, and often appear more or less abnormal. Those found in thick mucus are frequently aflagellate, and are only recognizable as trichomonads by the characteristic movements of the undulating membrane. When this monkey is menstruating, and blood is present in her vagina, the trichomonads sometimes ingest the red corpuscles; but at other times they contain nothing but bacteria.

Although this macaque has now been vaginally infected with *Trichomonas*

[1] These were all made at times when the vaginal secretions were difficult to study: cf. p. 543 *supra*.

for over 3 years, she appears to have suffered no ill-effects whatsoever from the "parasite." It has produced no recognizable pathogenic effects. Her vaginal secretions are still in no way abnormal, and I have no reason to believe that the flagellates have produced any lesions in her genitalia. Squamous cells, leucocytes, and red corpuscles are normally present—in varying numbers, at different stages in the menstrual cycle—in Susanna's discharges: but such cellular constituents are, of course, typical of the normal vaginal exudate in primates. I have been able to detect no difference in their abundance or character since the experimental introduction of *Trichomonas*. Artificial infection with this flagellate has certainly caused no vaginitis, endometritis, or other recognizable inflammatory condition: and if any competent worker were now to study Susanna—in ignorance of her history—he would undoubtedly pronounce her to be infected with a typical "*T. vaginalis*" associated with no evident pathological symptoms of any sort.

(8) *Further observations on an intestinally uninfected Macaque experimentally infected* intra vaginam *with* Trichomonas *of intestinal origin*

After I had succeeded—as just described—in establishing an intestinal strain of *Trichomonas* in Susanna's vagina, I examined her faeces again with great care. At the time of her vaginal infection she was uninfected intestinally: moreover, I had already found that the species of *Trichomonas* used in this experiment was incapable of living permanently in her gut[1]. But all macaques have filthy customs, and thus expose themselves habitually to every kind of infection. They often defaecate and urinate while sitting down, and remain unconcernedly seated in the resultant mess; and when they then get up and move about, their perineums may remain plastered with a mixture of faeces, urine, and vaginal secretion for hours. Every opportunity is therefore afforded for interchange of intestinal and genito-urinary infections. Furthermore, Susanna—like all other female macaques that I have studied—has the habit of eating her vaginal mucus and menstrual discharges[2]; and as these now contain trichomonads, she is continually making the unconscious experiment of reinfecting herself *per os*. I imagined, therefore, that a new intestinal infection might occur sooner or later, and have always been on the look-out for such an event. To the best of my belief, however, Susanna is still uninfected intestinally.

Nevertheless, all the microscopic and cultural examinations of Susanna's faeces, since she acquired her vaginal infection, have not been negative: some have been positive for *Trichomonas*, and as my interpretation may be wrong I must record my actual findings more precisely. Their significance is debatable.

Since I infected Susanna with *Trichomonas* intravaginally (21. ii. 31) I

[1] Cf. p. 554 *supra*.

[2] Other macaques—both male and female—also appear to relish these humanly unsavoury excretions.

have examined her faeces—microscopically and culturally every time—on 95 several days up to the time of writing (June, 1934). Of the direct examinations 88 were negative and 7 positive; while cultures were negative 70 times and positive 25 times.

I first rediscovered trichomonads in this monkey's faeces on 7. iii. 31 (a fortnight after vaginal inoculation): and I then recovered them in cultures only—the microscopic examination being negative. Afterwards I found them from time to time—usually in cultures—and with great irregularity. In the majority of cases I was able to convince myself that the positive findings were due to accidental contamination of the faecal specimens with vaginal secretion. It is, indeed, by no means easy to obtain certainly uncontaminated samples of faeces for examination. Stools passed spontaneously are often grossly and obviously mixed with vaginal mucus, and special precautions have to be taken to obtain fresh and clean specimens. When the faeces have been certainly or even probably contaminated with vaginal secretion, I have almost invariably succeeded in recovering *Trichomonas* in cultures (and occasionally in finding the flagellates by direct microscopic examination): but when I have taken stricter precautions, and have made sure that the faecal samples were uncontaminated, I have usually failed to detect trichomonads by any method of study. As a general rule, Susanna's faeces have remained negative for *Trichomonas* when all possibility of vaginal contamination has been rigidly excluded.

On a few occasions, however, I have recovered *Trichomonas* from Susanna's faeces under conditions which rendered all direct contamination from her vagina absolutely impossible. The microscopic and cultural examinations were both positive; and there could be no doubt that the flagellates were, at the moment, actually present in her rectum. I attribute these rare positive examinations to two causes: (1) occasionally the flagellates migrate into the hind-gut from the vagina, and remain there for a variable time; and (2) occasionally a few living trichomonads may reach the rectum when they are swallowed with the vaginal discharges. These not unreasonable suppositions would readily account for all my positive findings, without assuming the existence of a true intestinal infection.

I have already noted elsewhere[1] that other intestinal protozoa of macaques may migrate into the vagina—though they cannot establish themselves in this situation—and I see no reason to believe that immigration cannot occur in the opposite direction. In my opinion, Susanna is now permanently infected with *Trichomonas* in her vagina, but not in her intestine—notwithstanding that the flagellates are sometimes demonstrable in her faeces. My interpretation may

[1] Dobell (1933). *E. histolytica*, *E. nana*, and other motile organisms can sometimes emigrate from the anus to the vagina; and even *Blastocystis* may sometimes be recovered in cultures of vaginal contents. This probably occurs also in human beings: for in a recent paper Green-Armytage (1931)—describing conditions in India—mentions the finding of "streptococci, *B. coli*, fungi, protozoa, trichomonas or even the ova of worms in the majority of cases of virginal leucorrhoea."

be wrong: but the distribution of positive and negative examinations since her vaginal infection is entirely unlike anything I have seen in any monkey or man with an ordinary intestinal infection. For periods of weeks and even months at a stretch, Susanna's faeces (when unsoiled with other dejecta) have been consistently negative after exhaustive examination. Moreover, it must be remembered that I had shown, by previous experiment, that the strain of *Trichomonas* concerned was unable to live permanently in this monkey's bowel.

Summary of all experiments

It will be convenient at this point, before proceeding to a more general discussion of the findings, to summarize all the experiments recorded in the eight preceding subsections. Briefly the results are as follows:

(i) An attempt to infect a *Macacus rhesus* by feeding it with *Trichomonas* isolated from a monkey of the same species failed completely.

(ii) Four similar attempts to infect a *Macacus sinicus* with the same *Trichomonas* (from *M. rhesus*) were equally unsuccessful.

(iii) Two attempts to infect another *M. sinicus* by intra-intestinal inoculation with the same flagellate were likewise wholly negative.

(iv) One attempt to infect a man *per os* with the same strain of *Trichomonas* (from *M. rhesus*) did not succeed.

(v) A strain of *Trichomonas* from Man, when fed to a *Macacus rhesus*, produced no infection.

(vi) The same strain (*T. hominis*) fed to a *Macacus sinicus* likewise failed to infect.

(vii) A strain of *Trichomonas* from *Macacus nemestrinus*, however, when fed to *M. rhesus*, gave rise to a temporary infection lasting about a month.

(viii) This *Trichomonas* (from *M. nemestrinus*, after passage through *M. rhesus*) produced, nevertheless, a permanent infection—lasting $4\frac{1}{2}$ years to date—when fed to a man.

(ix) The same strain of *Trichomonas* (from *M. nemestrinus*), after passage through *M. rhesus* and Man, when fed to a *Macacus sinicus* gave rise to an infection which endured for approximately $1\frac{1}{2}$ months and then died out. But

(x) The very same *Trichomonas* (which was derived from *M. nemestrinus*, and which produced a temporary intestinal infection in *M. rhesus* and *M. sinicus*, and a permanent intestinal infection in Man) when introduced into the vagina of *M. sinicus* established itself enduringly as a typical "*T. vaginalis*." The infection has now persisted for some $3\frac{1}{3}$ years.

(xi) The monkey (*M. sinicus*) in which a permanent vaginal infection with an intestinal *Trichomonas* was established, has probably since reacquired no permanent intestinal infection—despite every natural opportunity for such reinfection.

(xii) No macaque suffered any harm whatsoever as a result of inoculation with any strain of *Trichomonas*—whether successful or unsuccessful.

(xiii) The one man successfully infected with *Trichomonas* (from *M. nemestrinus*) has likewise remained normal ever since.

In order to make the foregoing infection-experiments clear, and for the reader's convenience, I give a summary of the relevant data in Table II.

Table II. *Summary of all attempts to infect Macaques and Man intestinally with* Trichomonas *from various sources*

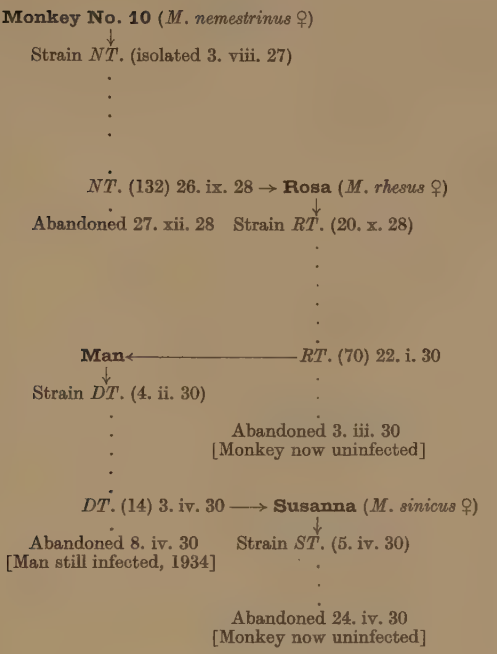
Experimental animal	Date of experiment	Strain of *Trichomonas**	Origin of strain	Mode of inoculation	Result
Jacko (*M. rhesus*)	28. viii. 25	*T.* (30)	*M. rhesus*	*per os*	No infection
Mungo (*M. sinicus*)	13. viii. 25	*T.* (29)	*M. rhesus*	*per os*	No infection
	22. viii. 25	*T.* (29)	,,	,,	,,
	4. ix. 25	*T.* (32)	,,	,,	,,
	2. iii. 26	*T.* (63)	,,	,,	,,
Rosa (*M. rhesus*)	26. ix. 28	*NT.* (132)	*M. nemestrinus*	*per os*	Infected for 1 month. Infection then died out
	2. iv. 30	*FT.* (373)	Man	,,	No infection
Susanna (*M. sinicus*)	13. viii. 25	*T.* (29)	*M. rhesus*	*per anum*	No infection
	22. viii. 25	*T.* (29)	,,	,,	,,
	26. ix. 28	*FT.* (234)	Man	*per os*	,,
	3. iv. 30	*DT.* (14)	*M. nemestrinus*, passed through *M. rhesus* and Man	,,	Infected for 1½ months. Infection then died out
Man	31. viii. 25	*T.* (31)	*M. rhesus*	*per os*	No infection
	22. i. 30	*RT.* (70)	*M. nemestrinus*, passed through *M. rhesus*	,,	Persistent infection (4½ years to date)

* The numbers in parentheses denote the serial generations of the subcultures used in the experiments.

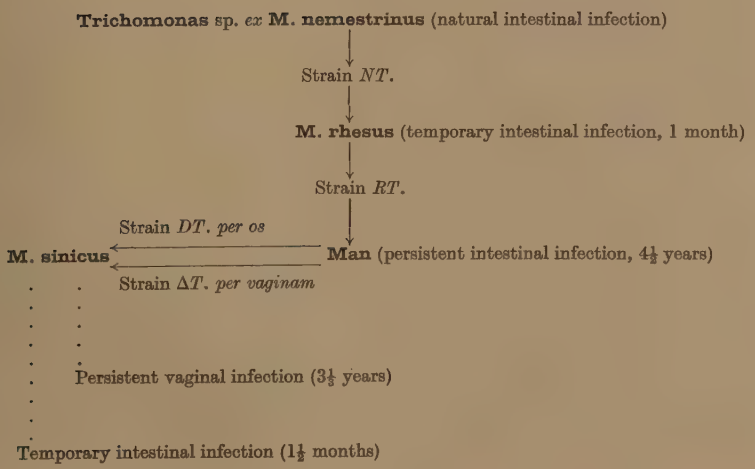
I also give a graphic synopsis of the results obtained with Strain *NT.* (and its derivatives) in the accompanying *Scheme A*. This summarizes all my positive experiments in the briefest manner possible, but takes no account of experiments which were negative. *Scheme A*, however, shows only the results of my attempts to induce intestinal infections with *Trichomonas*; but as I made a further (successful) attempt to produce a vaginal infection in one macaque, I have exhibited the history of this experiment in an additional scheme (*Scheme B*).

If the reader will inspect these schemes and Table II—referring, if need be, to Table I, p. 540—I think he will have no difficulty in comprehending all my experiments and results.

Scheme A



Scheme B



IV. Discussion

I shall now attempt to reconcile the apparently discordant results related in the earlier pages of this paper, and bring them into line with certain no less inconsistent findings recorded by other workers. Almost every experiment I made produced a result different from that anticipated—in most cases one diametrically opposed to my expectations: but I am confident that my main findings are correct—despite their seeming discrepancy—and the very fact that nearly all my results disagreed with my anticipations should guarantee their objectivity.

It may now be taken for granted that the trichomonads of most mammals gain access to new hosts in an unencysted state. Unlike other intestinal protozoa, they do not encyst at any stage in the life-cycle—the free flagellate form being the only one found. But the flagellates—again unlike most others—are extraordinarily resistant: they can remain alive at ordinary room-temperatures (under otherwise favourable conditions) as long as the cysts of other intestinal protozoa, and they can—like cysts—pass unscathed through the mouth and stomach of their new host to their final destination in the bowel. All this is now well known for trichomonads in general, and there is no reason to suppose that those of the Primates are in any way exceptional.

In the present experiments I have, indeed, shown that the ingestion of active trichomonads may produce a solid infection. When I swallowed *Trichomonas* (Strain *RT.*) myself, I acquired an infection which has now endured for some $4\frac{1}{2}$ years. Consequently, there is no reason to suppose that my method of experimentation was at fault. My "failures," in other cases, must be accounted for in other ways.

Nearly all "successful" results previously reported by other workers—for other trichomonads and different hosts—have been made by another method. Animals have usually been fed on faeces containing the flagellates—not on cultures: but the fact that I have, purposely, always experimented with "pure" cultures can hardly be urged as an objection against my findings. By using cultures only, I have diminished the unknown factors. My method is a refinement of that previously employed, and should therefore yield more certain—not less certain—results.

It might, however, be urged that in attempting to infect monkeys with trichomonads which had been propagated *in vitro* before they were inoculated, I defeated my own objects. It might be suggested that *Trichomonas* loses its infectivity on cultivation—as other protozoa are known to do—and that this is a sufficient explanation of all my "negative" experiments. But such objections have little weight; for there is no known instance—to the best of my belief—of any intestinal protozoon having ever lost its infectivity for its natural hosts as a result of prolonged cultivation *in vitro*. The only case in which a loss of infectivity has been, as yet, established, concerns *E. histolytica*. I have shown[1]

[1] Cf. Dobell (1931).

that this amoeba may, on continued cultivation outside its hosts, lose its infectivity for cats: but the cat is not a natural host, and in the same series of researches I demonstrated that continuous propagation *in vitro*—even for as long as 5 years—neither annulled nor abated the power of this parasite to infect two of its natural simian hosts (*M. rhesus* and *M. sinicus*). That a hypothetical "loss of infectivity" due to cultivation can explain my present "failures" is also contradicted by some of the experiments just recorded. Although I certainly failed[1] to infect Mungo (*M. sinicus*) with a *Trichomonas* from *M. rhesus* cultivated for a period of some $3\frac{1}{4}$ months (29 generations), I easily infected myself with another strain from *M. nemestrinus* (passed through *M. rhesus*) kept continuously *in vitro* since isolation for 15 months (70 cultural generations). My own findings therefore afford no support for the view that *Trichomonas* may lose its infectivity for its natural hosts through continued extracorporeal cultivation, and I can find no evidence in favour of such a suggestion in any publications of others. Consequently, I consider it reasonable to conclude that *T. hominis* (and its allies in macaques) suffers no loss of infectivity for its natural hosts on continued cultivation *in vitro*. It is for those who take the opposite view to produce evidence for their belief.

Another point of importance—one which has an obvious bearing on all such experiments—is the ability of *Trichomonas* to resist concentrations of hydrochloric acid similar to those which it must encounter in its passage through the stomach. If the flagellates enter the bowel *via* the mouth and stomach, they must be able to withstand the gastric secretions for a time sufficient to permit their safe passage: and it thus seems reasonable to suppose that infection would be most readily acquired by men or monkeys whose gastric juice is deficient in acid, and, conversely, that those strains of *Trichomonas* which are most resistant to hydrochloric acid would have the best chance of getting through the stomach and so arriving at their intestinal destination.

When I began these researches very little was known about the tolerance of *Trichomonas* to hydrochloric acid, or about the gastric acidity of monkeys or men infected (or infectible) with this flagellate. Levy (1922), however, had noted that human infection with trichomonads was sometimes associated with hypochlorhydria; and Tsuchiya (1925) soon afterwards adduced additional evidence[2]. Some American workers apparently believe that hypochlorhydria is an effect of *Trichomonas*-infection (for they regard the flagellate as pathogenic): but to me it seems far more probable that the condition is a contributory cause. Those people whose gastric juice is most deficient in acid are obviously the least resistant to invasion—assuming that hydrochloric acid is harmful to trichomonads.

The assumption seems clearly justifiable; but curiously enough nobody

[1] This failure was thrice confirmed in later experiments: *vide* Table II (p. 562).

[2] The fact—if it be one—was noted even earlier by various workers: for example, Brumpt (1913) refers to it more than once.

appeared to have made any exact experiments to verify it when I began the present work. It was therefore necessary to test my own strains of *Trichomonas* in order to ascertain their powers of tolerating hydrochloric acid, and to find out whether any differential resistance existed between strain and strain. I entrusted this investigation to Miss Ann Bishop, who published her results in 1930. She showed that my strains *T.*, *FT.*, and *NT.*, were all unable to withstand *N*/20 HCl for more than a very short time at 37° C. (usually no longer than 5 minutes); but that Strain *T.* was the least resistant, and Strain *NT.* the most resistant *in vitro*.

These last findings might be taken to "explain" some of my results, but they afford no complete explanation. For example, I never succeeded in infecting *M. sinicus*, *M. rhesus*, or Man, with Strain *T.* inoculated *per os* (6 attempts with 3 subjects); whereas I was able to infect the same species, in the same way, with Strain *NT.* or its derivatives (3 experiments on 3 subjects). As *NT.* was more resistant than *T.* to hydrochloric acid this might seem significant: but the fact that the infections produced in *M. rhesus* and *M. sinicus*, with Strain *NT.*, were temporary and not permanent (*ca.* 1 month and $1\frac{1}{2}$ months respectively), indicates clearly that the ability of the trichomonads to resist gastric juice does not determine their subsequent establishment in the intestine. Both Rosa and Susanna were infected with Strain *NT.* for a time—proving that the flagellates passed safely through their stomachs—but nevertheless both their infections died out later. In my own case, the same strain survived passage through my stomach and then established itself permanently in my intestines. Permanent infection obviously depends, therefore, upon some factor, or factors, other than the HCl-content of the host's stomach: though it is equally obvious, of course, that strongly acid gastric juice would form a good defence against infection with any strains of *Trichomonas* highly sensitive to acid.

Additional evidence that infection, or non-infection, in the present series of researches, is not explicable along such lines is supplied by my other experiments with Strain *T.* I failed to infect *M. sinicus* and *M. rhesus* and Man with this strain *per os*, but I also failed equally to infect *M. sinicus* (2 attempts with the same monkey) by inoculation *per anum*. The possible effects of gastric acidity were here eliminated, but the results were likewise completely negative.

I shall return to this subject of infectibility later, and must now consider very briefly another problem on which my experiments were designed to throw light—the question of the pathogenicity of *Trichomonas*. Many people still believe that this flagellate is a dangerous parasite, capable of causing diarrhoea and dysentery, with ulceration of the intestines, and even abscesses and lesions in various other parts of the body. The fact that *Trichomonas* will ingest red blood-corpuscles—when they are available—is often regarded as a proof, or at least a confirmation, of its pathogenic propensities[1]. Certain

[1] That this is not so was pointed out long ago (Dobell and O'Connor, 1921, p. 91), and has since been reaffirmed by several other writers.

writers also regard the 5-flagellate forms ("*Pentatrichomonas*") as particularly deadly, and even exalt them to the status of a separate species ("*P. ardin-delteili*").

The evidence for such beliefs has always seemed to me extremely unsatisfactory[1]. I have never been able to discover any convincing arguments to show that any *Trichomonas* of men or monkeys is pathogenic; and everything that I have yet seen with my own eyes, or read in the works of others, induces me to believe that the "pathogenicity" of these flagellates is a myth. The mere fact that most macaques are naturally infected indicates that the infection is harmless. I know, moreover, from my own experiments—not only with *Trichomonas*, but also with *E. histolytica*, *E. coli*, *Dientamoeba*, and other intestinal protozoa—that ability to ingest red corpuscles is no criterion of criminality: it is a mutable faculty, in certain intestinal protozoa, and not an index of pathogenicity[2]. Furthermore, I cannot regard "*Pentatrichomonas*" as a distinct genus or "*P. ardin-delteili*" as a good species zoologically.

I admit that in all these views I may be wrong; but at all events I have the courage of my convictions, and I have now put them to the proof on my own person. When I attempted (for the second time) to infect myself with a *Trichomonas* derived from a monkey, I purposely selected for trial a strain which ingested my own red corpuscles with particular avidity *in vitro*, and which was characterized by the frequency of 5-flagellate individuals ("*Pentatrichomonas*"). The result of the experiment fulfilled my hopes. I infected myself with this bloodthirsty strain at the first attempt, and I have remained infected for $4\frac{1}{2}$ years without suffering the least inconvenience[3].

The same is true of the monkeys which I have been able to infect with *Trichomonas* intestinally, though here the evidence is less convincing: for I succeeded with only two animals (Rosa and Susanna), and in both the infection persisted for only a few weeks. Nevertheless, the results, as a whole, confirm my belief that the intestinal trichomonads of men and macaques are entirely harmless to their hosts. No disease has been produced in any of my animals as a consequence of experimental infection.

All my experiments were made with "pure" cultures of *Trichomonas* which contained, of necessity, numerous unknown bacteria also. Some of these might well have been pathogenic, but I was unable to test them all in detail. However, since none of my inoculations produced any clinical symptoms—such as diarrhoea or dysentery—in any experimental animal, I conclude that not only the trichomonads but all the other micro-organisms in my cultures[4] also were normally harmless to healthy men and macaques.

[1] Cf. Dobell and O'Connor (1921), especially pp. 89–93. See also Reichenow (1925).

[2] Cf. Dobell (1931), p. 55 *sqq.*

[3] Cf. p. 553 *supra*.

[4] The only strain about which I felt any apprehension was Strain *T*. This, as the reader may recall, was derived from a dead Rhesus monkey which died of unknown causes. Before attempting to infect myself with it, however, I had inoculated this strain *per os* and *per anum* into 3 of my monkeys without any harmful consequences. The risk of injuring myself therefore appeared negligible.

Unfortunately I have had no opportunity of examining any experimental animal in my series *post mortem*. The only infected monkeys which came to necropsy were No. 9 (*M. rhesus*) and No. 10 (*M. nemestrinus*), from which I obtained Strains *T.* and *NT.* respectively. Both were naturally infected with *Trichomonas* and many other intestinal protozoa (including *E. histolytica*). The Rhesus monkey had been too long dead for any satisfactory histological study of her intestines to be feasible: the Pigtail showed no intestinal lesions of any sort. I am aware, of course, that other workers have found trichomonads in the tissues of the bowel *post mortem*[1], and have usually regarded this finding as a proof of pathogenicity: but since these flagellates can remain alive and active so long at ordinary room-temperatures, it seems to me far more probable that in all such cases they had merely penetrated the gut wall after death. The absence of any tissue-reaction in all similar instances yet described supports this interpretation. I have been able to find no account of an intestinal lesion of any sort which could be attributed to *Trichomonas* with any plausibility. Conclusive evidence that the trichomonads of men or monkeys can invade or injure the intestine during the life of the host appears to be still lacking: and the evidence hitherto advanced to prove the pathogenicity of *Trichomonas hominis* for other mammals appears, to say the least, unsound.

Too much has already been published on this subject for a full discussion of all the records to be possible here; and they are really, in the present connexion, irrelevant. I must, however, refer briefly to the recent experiments made with kittens by Kessel (1928 *a*). He found that these animals, when naturally infected with *Trichomonas*, suffer from diarrhoea or dysentery which is usually fatal. They can also be infected experimentally with *T. hominis* (from Man)[2] and a similar flagellate from "the monkey" (*M. rhesus*)—with the same pathological results. Though Kessel worked with "pure" cultures of *Trichomonas*, all his strains were—like mine—mixed with numerous unknown bacteria, whose possible pathogenicity for kittens was not determined: and this obvious source of error was therefore not eliminated—notwithstanding his statement that, in his experiments, "Koch's postulates have been in the main fulfilled." It is hardly conceivable that Koch himself would have sanctioned so strange an interpretation of his rigid requirements[3], and consequently I cannot regard Kessel's evidence as conclusive—especially as it disagrees with all my own experience with the trichomonads of men and macaques. But I have made no serious study of *Trichomonas* in kittens; though I may note that the only kitten which I have yet found to be naturally infected was normal, and my only attempt to infect another with *T. hominis* was unsuccessful[4].

Since there is no convincing evidence to prove that the trichomonads of men and macaques infect or affect the tissues of the body during life, there is

[1] The best human case known to me is that reported by Wenyon (1920).

[2] This is not in agreement with the work of Hogue (1922) and several other experimenters.

[3] When Koch demanded "pure cultures" he meant cultures which were really pure; and he would certainly have been dissatisfied with those merely "pure in the main."

[4] Unpublished observations.

no reason to believe in the existence of any acquired immunity in animals which have recovered from infection. No protozoon which lives on the contents of the bowel—not upon the bowel itself—has ever been shown capable of immunizing its host against subsequent infection: and it is hard indeed to conceive of any natural mechanism which could cause such immunity. Accordingly, I do not believe that any of the animals used in my experiments were unsuitable for further trials after a first inoculation had failed. If it be said that I probably rendered some of my subjects immune by unsuccessful attempts at infection, and that my later experiments with the same hosts are therefore invalid, I ask for concrete evidence—which has not yet been published—in support of such objections. And I would point out, in confirmation of my own views, that I have personal evidence to the contrary. In 1925 I inoculated myself unsuccessfully with Strain *T.* of *Trichomonas* (from *M. rhesus*); but this did not in the least hinder me from infecting myself—pretty permanently—in 1930 with Strain *RT.* (from *M. nemestrinus* and *M. rhesus*). It will be time, I think, to talk about "acquired immunity to *Trichomonas*" when we have some definite facts for discussion. At present we have none. "Natural immunity"—perhaps better called "uninfectibility"—is, of course, another matter, and I shall consider it presently.

My experiments with *Trichomonas* have taught me that certain macaques may be not only uninfected but also uninfectible—even with strains of these flagellates proper to their own species. Macaques may also acquire infections which are only temporary, and these may sometimes be discoverable only by most careful investigation. A few casual "positive" or "negative" examinations mean very little, and all experiments are worthless unless they have been made with thoroughly-studied animals and followed up for long periods in great detail. Modern methods of cultivation, however, increase the value of all examinations enormously, and may frequently enable an infection to be detected when it would otherwise be overlooked.

To emphasize and illustrate these points I may recall two of my experiments. Rosa (*M. rhesus*) and Susanna (*M. sinicus*) were both subjected to exhaustive examination before, during, and after my attempts to infect them with *Trichomonas*. The first monkey acquired an infection (with Strain *NT.*) which persisted for about a month, and then died out: yet had I made daily microscopical examinations only—and not cultures as well—the experiment would have been registered as a clean "negative." No infection would ever have been detected. On the other hand, if I had been fully satisfied with the results of all cultural examinations for 5 weeks following inoculation, I should have been able to record a definite "positive." The monkey was then infected with *Trichomonas*, readily recoverable in cultures every day, and there was no reason to suppose that her infection would not endure.

In the case of Susanna, I easily infected this monkey with Strain *DT.*, and *Trichomonas* was readily demonstrable microscopically (and culturally) in her faeces for more than 5 weeks after inoculation. Had I ended the experiment at

this stage, it would have made a good "positive": but in reality it was not. Like that made with Rosa, the experiment really proved—when properly followed up—that no permanent infection had been established.

I have not succeeded in infecting any macaque, as yet, with *Trichomonas* of purely human origin. Kessel (1928), however, claims to have done so, and his findings must therefore be considered here. According to his published records[1], he obtained 2 macaques (No. 3, *M. irus* [=*cynomolgus*] and No. 5, *M. lasiotis*) which were naturally uninfected with *Trichomonas*. The evidence for their non-infection is that they were "examined[2] three times a week from January 2 to March 8, 1924," and found to be "negative." This is pretty good evidence—assuming that the monkeys did not always pass formed faeces, and that always fresh samples were examined by a competent examiner[3]. Kessel then fed both of these macaques on "5 c.c. of dilute human feces" containing various mixtures of protozoa, including *T. hominis*, and adds merely that "The routine follow-up examinations were made from three to six weeks after the feeding," and *Trichomonas* was then "found to have been established." According to his own Table 4, however, the *M. cynomolgus* was still negative "after feeding[4]."

On such evidence I am not convinced that Kessel succeeded, or did not succeed, in establishing a permanent infection with *T. hominis* in either *M. cynomolgus* or *M. lasiotis*. In the light of my own experience, his records—as published—appear to prove nothing. I am therefore unable to accept his wider generalization[5] that in his work "Monkeys were experimentally infected with...*Trichomonas hominis* of man." There is no clear proof that even a solitary macaque was solidly infected.

Reichenow (1925) mentions that he was able to infect himself with a cultivated strain of *T. hominis* (from Man), but unfortunately gives no evidence for his statement. In the absence of all essential data[6] this claim cannot profitably be discussed. It appears, however, to be the only record of an experimental human infection with *T. hominis* (of human origin).

I have also been able to find only a single record of any previous attempt to infect a human being with trichomonads of simian origin. Deschiens (1927, p. 21) states that he cultivated, from a chimpanzee, a *Trichomonas* (named "*T. anthropopitheci*," and "distinguishable by several morphological characters

[1] Kessel (1924) published an earlier account of the same work: but it does not agree in every detail with his later paper, so I assume this to be the correct version.

[2] It is not stated *how* they were examined, but apparently no cultures were made.

[3] The record gives us no assurance on these vitally important points.

[4] Kessel (1928), p. 294.

[5] Kessel (1928), p. 299.

[6] Further details were promised, but—apart from another brief reference (Reichenow, 1931)—I have sought them in vain in this author's later publications. I may add, however, that Reichenow apparently agrees with me (see Dobell and O'Connor, 1921)—though he omits to mention it—that the number of anterior flagella in "*T. hominis*" is no certain generic or specific criterion, and that the power of ingesting red corpuscles is no index of pathogenicity.

from *T. hominis*"[1]); and that a human volunteer ingested a culture (30th generation, but no dates recorded) with negative result. Insufficient details are given for any interpretation of this experiment to be yet possible[2].

Trichomonads ("*T. vaginalis*") are well-known inhabitants of the human vagina and male urethra. Similar organisms were first reported from the vagina of a macaque (*M. rhesus*) by Hegner and Ratcliffe in 1927, and named "*T. macacovaginae.*" The flagellates were found in 3 monkeys—all infected with *Trichomonas* intestinally. No evidence was given to prove that this "new species" was different from the intestinal species present in the same monkeys; that it actually inhabited—and had not merely strayed into—the vagina; or that it differed from "*T. vaginalis*" or "*T. hominis.*" A little later, however, Hegner (1928) announced that he had achieved the "experimental transmission of trichomonads from the intestine and vagina of monkeys to the vagina of monkeys (*M. rhesus*)": but the evidence for his claims again appears wholly inadequate. None of the monkeys used was examined sufficiently, and information vital for the interpretation of the experiments was not obtained (or, at least, not published). For example, we are not informed whether or no the macaques inoculated *per vaginam* already harboured intestinal trichomonads; but as they belonged to a colony in which intestinal infections were common[3], they probably did. If so, this greatly increased the chances of error. It would take too much space to discuss these findings in detail; but no discussion is needed, as Hegner himself admits that "The difficulties involved in work of this type were found to be very great, so great indeed that the data obtained are published herewith in preliminary form since more satisfactory material must be available to make further work of any great value[4]." (One wonders why this author considered "more satisfactory material" only necessary for "further work.")

To add to the confusion, the same writer has since recorded the finding of *T. vaginalis* in wild specimens of *Macacus philippinensis*[5] and captive Chimpanzees[6]. In the latter case, the flagellate was so named simply because "the species described from chimpanzees do not exhibit morphological differences sufficient to separate them specifically from those that occur in man." Unfortunately, it is now becoming fashionable to call all the intestinal protozoa of "monkeys" by the names of the corresponding forms found in men—merely because they are not obviously different in structure. Hegner

[1] Hegner and Chu (1930, p. 83) say that "these trichomonads seem to possess no morphological characteristics by means of which they can be separated from human intestinal trichomonads": but they adduce no evidence in support of this contradiction of the statement made by Deschiens.

[2] Deschiens (1927) states that apparently confirmatory attempts at cross-infection were in progress when he wrote, but I have gleaned no further information about them from his later publications.

[3] Cf. Hegner and Ratcliffe (1927).

[4] Hegner (1928), p. 261.

[5] Hegner and Chu (1930). The flagellates were found "in 2 of the 44 monkeys": but as only 16 of the 44 were females, and the genito-urinary organs of the 28 males were apparently not explored the ratio seems inexact.

[6] Hegner (1934 *a*).

and Chu (1930), for example, call the *Trichomonas* of *Macacus philippinensis* "*T. hominis*": their only reason for so doing being, it seems, that "measurements[1] furnish no evidence that human and monkey intestinal trichomonads belong to separate species." This is a very easy way of settling a large number of problems; but it is surely not very scientific.

It will be evident that Hegner has now stated, or implied, that the vaginal and intestinal trichomonads of *Macacus rhesus* are specifically different, but has published "experiments" to show that they are identical: while he has also expressed the opinion—directly or indirectly—that "*T. vaginalis*" is, and is not, the same species as "*T. hominis*"; and that both naturally inhabit the intestine and the vagina of macaques, chimpanzees, and human beings. Every eventuality thus appears to have been anticipated; evidence alone seems lacking to prove anything.

In a paper just published in abstract, Kessel (1933) has brought forward new evidence to show that the intestinal and vaginal trichomonads of Man are specifically distinct. He had previously demonstrated—to his own satisfaction[2]—that *T. hominis* is readily transmissible to kittens. Now he has attempted to infect these animals intestinally with *T. vaginalis* (of human origin). All his efforts were seemingly unsuccessful, so he concludes that the two forms are probably not identical. But this is obviously a roundabout way of attacking the problem, and any results so obtained are bound to be ambiguous; for it can never be determined whether or no the vaginal trichomonads of Man can live in the human intestine, and *vice versa*, by attempting to implant them into cats. Experiments of this sort really add new and needless complications to a riddle capable of solution by simple and direct methods.

In the recorded work of others I have found nothing to help me in the interpretation of my own findings, while some of my observations and experiments appear—at first sight—contradictory. For instance, I entirely failed to infect any macaques permanently with trichomonads derived from other macaques or from Man, though I successfully infected a man (more or less permanently) with similar flagellates obtained from a macaque. Yet no other worker, so far as I can discover, has ever had any greater success with similar experiments: and nobody has yet proved conclusively that any *Trichomonas* derived from any monkey or man can be experimentally established in any other monkey or man. I shall therefore say nothing more about the work of others, but endeavour to analyse my own results in further detail—beginning with my attempts at producing intestinal infections.

[1] Their measurements, moreover, are extremely unsatisfying, though presented with great show of precision. Even allowing for the fact that they are based on only 100 specimens, they yield the astonishing result that *T. hominis* (of Man) has an average length of "$6{\cdot}14\mu \pm 0{\cdot}02\mu$," while the simian form is still smaller ("$5{\cdot}94\mu \pm 0{\cdot}08\mu$"). In my experience the normal flagellates are about twice this length.

[2] Kessel (1928*a*). Hogue (1922), Brumpt (1925), and others, have denied that cats are intestinally infectible with *T. hominis*. They regard "*T. felis*" as a separate species.

If the intestinal trichomonads of men and macaques all belong to one species—and I can find no constant feature to distinguish any of them morphologically—then it follows that they should all be interchangeable, between host and host, like human and macaque strains of *E. histolytica*[1]. The fact that I have found such interchange usually impossible might therefore be taken to constitute a *prima-facie* case against the specific identity of the strains which I have used. It might be thought that I failed to infect *M. rhesus* and *M. sinicus* permanently with the *Trichomonas* of *M. nemestrinus*, and also with that of Man, simply because the flagellates in these various hosts belong to several distinct species—each proper to its own host, and none transmissible to any other. But it is impossible to uphold such a view in the face of other evidence—equally certain—proving (1) that a *Trichomonas* derived from *M. rhesus* (and found unable to infect *M. sinicus* and Man) could not infect another *M. rhesus*: and (2) that a *Trichomonas* from *M. nemestrinus* (incapable of establishing itself permanently in *M. rhesus* and *M. sinicus*) has been found readily transmissible to a human being, in whom it has now lived happily for several years.

It is clear that some strains of *Trichomonas* are more easily transplantable from host to host than are others: or, to put it another way, *strains differ in their infectivity*. This is shown by the following facts: (1) I never succeeded in infecting any animal (*M. rhesus*, *M. sinicus*, or Man) with Strain *T.* (derived from *M. rhesus*); whereas (2) all my attempts with Strain *NT.* (from *M. nemestrinus*), and its derivatives, were uniformly successful—more or less. The *infectivity* and *transmissibility* of any given strain are therefore factors of importance: but they are obviously not the only factors to be considered—otherwise Strain *NT.* should have been able to establish itself in other macaques as easily as it did in a man.

Another highly important factor must be taken into account. Not only do different strains of *Trichomonas*—of the same species—differ in their transmissibility and infectivity, but it seems equally clear that *all hosts are not equally infectible*. For reasons which are still obscure, certain individuals—both macaques and men—are "resistant" to infection, "naturally immune," "uninfectible," or capable of harbouring *Trichomonas* for only a short time. It is impossible to believe that my Strain *T.* could not infect *M. rhesus* simply because it could not live in Jacko, for it was isolated from a monkey of the very same species. My negative experiment merely showed that Jacko was a specimen of *M. rhesus* incapable of acquiring infection with a *Trichomonas* naturally occurring in monkeys of his own kind.

The following consideration further supports this interpretation. Macaques are gregarious; and their habits are so dirty and—to us—unhygienic, that all members of every family must be continually exposed to infection with every available species of intestinal protozoon. Every facility appears to exist for the acquisition of infections of every sort. Consequently, if any one individual

[1] Cf. Dobell (1931).

in a tribe were infected with *Trichomonas*, the infection would be expected to spread quickly to all other members in a state of nature[1]. It is not surprising, therefore, that Hegner (1929) found "almost 100 per cent.[2]" of wild macaques in the Philippines infected with trichomonads; and it is probably true that most macaques—wild or captive—of every species, everywhere, harbour *Trichomonas* in their intestines. No systematic search has yet been made, but the casual findings already reported furnish cogent evidence.

Accordingly, it seems probable that any macaques which are found to be naturally uninfected with *Trichomonas* are also uninfectible. They have no trichomonads in their intestines not because they have never been exposed to infection but because trichomonads cannot live in them permanently. It seems to me almost certain that my monkeys Jacko, Mungo, Susanna, and Rosa—all uninfected with trichomonads when they came into my possession—must have had many opportunities of acquiring infection before they were captured: and the very fact that they were not naturally infected indicates that they were not readily infectible. My experiments merely confirmed this; for I was unable to infect Jacko and Mungo at all, and succeeded in producing only transient infections in Susanna and Rosa with strains derived from hosts of different species.

In my earlier experiments with *E. histolytica* I adopted a different method of experimentation[3]. I cured various macaques of their natural infections with this amoeba before I attempted to reinfect them with strains from other sources. All my attempts were successful, in striking contrast with the present experiments with *Trichomonas*: and though at first sight these two series may seem discordant, the results are really in harmony. As every monkey which I infected with *E. histolytica* was already naturally infected (before I eradicated its infection with emetine), it follows that every monkey must have been infectible with this parasite at the time when I made my experiments.

With human beings the situation is probably very different. *Trichomonas* is comparatively uncommon in civilized Man, but many men are probably uninfected merely because they have never been exposed to infection—not because they are uninfectible. This explanation seems, at all events, to cover my own case. The ease with which I infected myself with Strain *RT.* showed that I was readily susceptible to infection by a suitable simian strain; and it seems unlikely, therefore, that I am incapable of infection with strains of human origin. Unfortunately no further tests of this sort are now possible.

Similar considerations are obviously applicable to the vaginal tricho-

[1] Deschiens (1927) believes that wild monkeys are less heavily infected than those kept in captivity, but I can find no reason to take this view.

[2] The actual figures were 37 out of 44 (Hegner and Chu, 1930)—approximately 84 per cent. However, the proportion is sufficiently high, and would doubtless have been higher if the monkeys had been examined more thoroughly. Cf. also Greig and Wells (1911), Branch and Gay (1927), etc.

[3] See Dobell (1931). This method cannot be used with *Trichomonas*, as there is no known means of eradicating infections with this organism.

monads. There is no good evidence to prove that *T. hominis* and *T. vaginalis* are specifically distinct. Growing in different environments, in different sites in the body, they may show slight morphological diversities; but it has been the general experience that, when cultivated in a similar manner, they are very closely alike. "Cultural forms of *T. vaginalis* are morphologically indistinguishable from *T. hominis*" (Andrews, 1929). It thus seems probable that the two forms are specifically identical, and that vaginal infection—like intestinal—may occur whenever an infectible host is exposed to infection with a suitable strain. My experiment with Susanna has shown that an intestinal strain of *Trichomonas*—derived from a macaque of different species, and capable of producing a typical "*T. hominis*" infection in a man—can live in the vagina of *M. sinicus* as a typical "*T. vaginalis*" for several years at least. The entire series of experiments with Strain *NT.* and its derivatives affords strong evidence, indeed, that the vaginal and intestinal trichomonads of men and macaques all belong to one and the same species[1]. In the absence of any good evidence to the contrary, I accept this conclusion for the present—notwithstanding my certain failure to transmit infections from one host to another on various occasions.

If my interpretation is correct, it follows that all further work with macaques along similar lines is futile. One can make experiments only with naturally uninfected animals[2]; but all such individuals—if one has the luck to find them—are worthless for experimentation. They are uninfectible, or capable of infection only temporarily and with difficulty; and any "negative" or "partly positive" results obtained can teach us nothing about the specific identity or diversity of any strains of *Trichomonas* derived from other monkeys or from men.

Useful experiments can alone be made, in future, on human beings: and until they are made—and made with every care and precaution—no absolutely final conclusions can be drawn. At present we can deal only with probabilities; yet these are, in some respects, already so great that I feel justified in summarizing the clear indications of all the evidence hitherto obtained as follows[3]:

General conclusions

(1) The intestinal and vaginal trichomonads of Man are not specifically different from one another, nor from the corresponding flagellates of similar habitat in the Macaques.

(2) Consequently, *Trichomonas vaginalis* Donné, 1837; *T. hominis* Davaine, 1860 [=*T. intestinalis* Leuckart, 1879]; and *T. macacovaginae* Hegner & Ratcliffe, 1927, are synonymous.

[1] If correct, this conclusion will involve some unfortunate changes in nomenclature—the name *T. vaginalis* having priority as designation of the intestinal trichomonads of Man.

[2] Monkeys "clean" from birth are as yet unobtainable; and in spite of innumerable claims there is still no known drug which will eradicate a *Trichomonas* infection in any animal.

[3] A summary of all my actual experiments has already been given on p. 561 *et seq.*

(3) Within this species, however, there exist diverse strains, distinguishable by minor morphological characters (size, average number of anterior flagella, etc.) and physiological properties (infectivity for various hosts, ability to ingest red blood-corpuscles, etc.).

(4) No strains are, *per se*, pathogenic to normal hosts—human or simian.

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THE INTESTINAL PROTOZOA OF A MUSKRAT, *FIBER* (= *ONDATRA*) *ZIBETHICA*, WITH A NOTE UPON *RETORTAMONAS* SP. FROM THE GUINEA-PIG

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(With 7 Figures in the Text)

The muskrat, *Fiber* (= *Ondatra*) *zibethica*, upon which the following observations were made was caught alive for Mr T. Warwick, B.Sc., of the Bureau of Animal Population, Oxford, at Montford Bridge, near Shrewsbury, on the River Severn, in February 1934. The animal was killed, and smears made from the contents of the caecum and colic loops were fixed by him in Schaudinn's solution containing 2·5 per cent. glacial acetic acid. On receiving these smears in 70 per cent. alcohol I stained them in iron haematoxylin. I wish here to record my indebtedness to Mr Warwick for sending me this material and for the trouble he has taken in obtaining and preparing it.

The present economic interest in the muskrat as a new member of the British fauna and in its migrations justifies the publication of these observations. Only one protozoan has been recorded from it hitherto: *Eimeria ondatrae-zibethicae* by Martin (1930). I failed to find Coccidia in the smears I examined, but the following four flagellates were present.

Trichomonas sp. (Fig. 1). This *Trichomonas* varied from 9 to 14μ in length. Its three anterior flagella were of equal length; approximately the same length as the body. The fourth flagellum, which ran along the edge of a strongly developed undulating membrane, projected freely beyond the posterior end. The axostyle was well developed and contained no granules. The siderophilic basal fibre was continuous and not made up of discrete granules as in *T. sanguisugae*. The nucleus was oval and contained several darkly stained granules embedded in a finely granular matrix. No distinct karyosome was visible. No cysts were found which could be attributed to this species.

Giardia sp. (Fig. 2). One specimen of this flagellate was found in the twenty films examined. It measured $11 \times 6\mu$. Four cysts were also found, measuring $9–9{\cdot}6 \times 5–6\mu$, but these were too faintly stained for a study of their detailed structure to be possible. A *Giardia* was described from the intestine of *Arvicola amphibius*, near Heidelberg, by Grassi and Schewiakoff (1888). The water vole is a near relation of the muskrat and very similar in habitat, but it is impossible to decide, upon the data available, whether the giardias from these different hosts belong to the same species.

Chilomastix sp. (Fig. 3). This flagellate was found frequently in all the preparations. It was almost spherical, measuring only 5–7μ, but the nuclear and cytostomial structures and the number and arrangement of the flagella were typical of the genus. There was no distinct karyosome. The deposit of chromatin on the inner side of the nuclear membrane was, in some individuals,

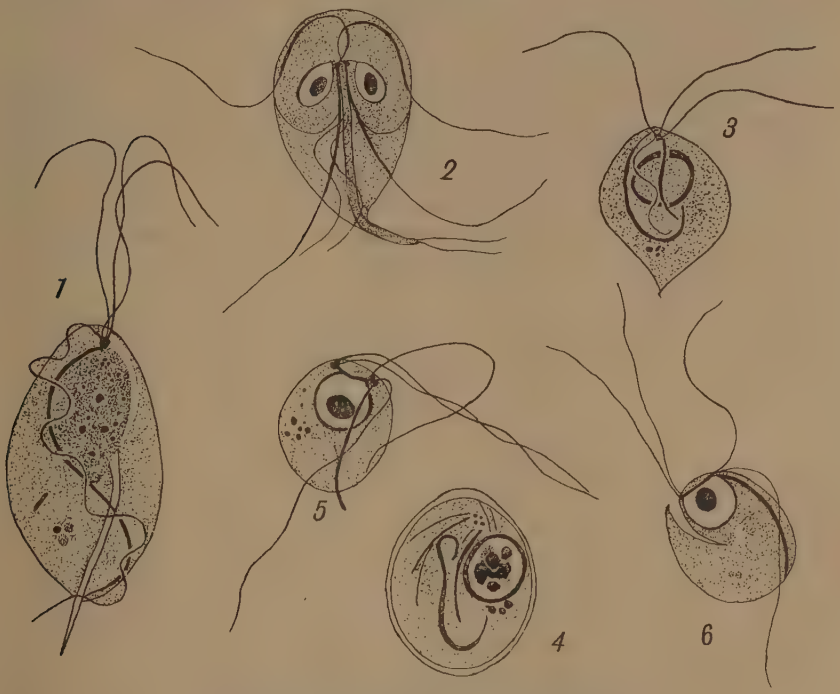

Fig. 1. *Trichomonas* sp. Fig. 2. *Giardia* sp.
Fig. 3. *Chilomastix* sp. Fig. 4. Cyst of *Chilomastix*.
Figs. 5, 6. *Retortamonas* sp.

heavier on one half the sphere than on the other. One individual was 13μ long, with elongated body distinctly pointed at the posterior end. Cysts (Fig. 4) measuring 6–8 × 4–5μ were frequent. The question arises whether these were the encysted stages of the small form of *Chilomastix*, in which case the flagellates themselves were usually as small or smaller than their cysts, or whether the cysts belonged to a larger strain of *Chilomastix* of which only one free individual was seen. No cysts smaller than 6 × 4μ were found.

Retortamonas sp. (Figs. 5 and 6). The flagellate found most frequently belonged to the genus *Retortamonas* and was very similar in structure to *Retortamonas rotunda* from *Bufo vulgaris* (Bishop, 1932). It was almost spherical and measured 4–8μ. It differed from *Retortamonas rotunda* in that the fourth, trailing flagellum was only slightly longer than the other three, and in

that the fine, siderophilic rays running from the nuclear membrane towards the karyosome were not visible. Tanabe (1933) has described a species of this flagellate, *Monocercomonas* (=*Retortamonas*) *lacertae* from the lizard *Erimias argus* Peters, and stated that a cytostome was often clearly seen, though not in all specimens. I saw no evidence of such a structure in *Retortamonas rotunda* nor in *R. orthopterorum*, but in a few specimens of *Retortamonas* from the muskrat I saw what appeared to be a distinct cytostome (Fig. 6). No dividing forms were found and no cysts.

The question of the nomenclature of this genus has been dealt with earlier (Bishop, 1932). Since that paper appeared Wenrich (1932) has maintained that *Embadomonas* is a synonym of *Retortamonas*. He believes that the generic name of *Monocercomonas* (which formerly was applied to flagellates similar to that which I have described (Figs. 5 and 6) from the muskrat) should be re-applied to this genus. This question was already discussed in another paper

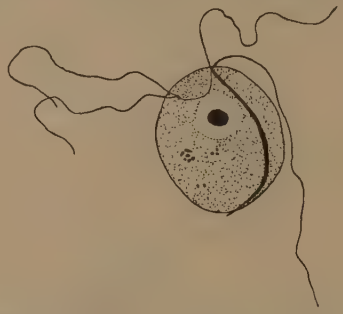

Fig. 7. *Retortamonas* sp. from the guinea-pig.

(Bishop, 1934) and I think that a change in the name of *Embadomonas* to *Retortamonas* is not necessary. The name *Retortamonas* is therefore still available for the genus to which this flagellate of the muskrat belongs.

A *Retortamonas* has been described from only one other mammal: *Cavia porcellus*, the Brazilian cavy. It was found by Cunha and Muniz (1921) and called by them *Monocercomonas caviae*, but renamed *M. hassalli* later (Cunha and Muniz, 1927) as the earlier name was shown to be preoccupied.

I have examined the caecal contents of four laboratory guinea-pigs and in two *Retortamonas* was present. This *Retortamonas* measured 6–10μ, was globular or slightly oval, and its four flagella were of equal length (Fig. 7). The structure of the nucleus was similar to that of the *Retortamonas* in the muskrat except that the nuclear membrane was much less evident and the whole nucleus is smaller in proportion to the body. In *Retortamonas* from both hosts the siderophilic axostyle was well marked. The thread joining the basal granules from which the flagella spring in pairs was very faint in the *Retortamonas* from the guinea-pig, whereas in the flagellate from the muskrat it was well developed. In the figure of *Monocercomonas caviae* (Cunha and Muniz, 1921) no thread is shown between the pairs of basal granules.

No attempt has been made to give the above-described flagellates specific names. Not only is detailed knowledge of morphology and method of division required before specific differences can be established, but cross-infection experiments of "clean" animals are also desirable before such points can be settled definitely.

SUMMARY

Flagellates belonging to four genera, *Trichomonas*, *Giardia*, *Chilomastix*, and *Retortamonas*, have been found in the caecum and colic loops of the musk-rat, *Fiber* (=*Ondatra*) *zibethica*.

A *Retortamonas* is described from the caecum of two laboratory guinea-pigs.

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ON THE EARLY STAGES OF *LESTODIPLOSIS ALVEI* BARNES (DIPTERA, CECIDOMYIDAE), ESPECIALLY IN RELATION TO THE LARVAL HEAD CAPSULE

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(With 15 Figures in the Text)

CONTENTS

A. Biology

The main object of this paper is the description of the larva of a new species of Cecidomyid, found by me in January 1933 in two old beehives from Selehurst, Horsham, Sussex. The adult Cecidomyids, reared from these larvae, were submitted to Dr H. F. Barnes, who has found them to be a new species described by him under the name of *Lestodiplosis alvei*, in the paper which immediately follows.

The bees had all died in both these hives, from some unknown cause, about four months previously, and the old nests had been kept in open wooden boxes in an outhouse during this time, and were in rather a dried up condition when handed over to me. Besides this larva, some pupae of *Aphiochaeta rufipes* (Diptera, Phoridae) were also found, as well as several Coleoptera, both larvae and imagines, and a few larvae of the wax moth *Meliophora grisella*, but by far the commonest inhabitants of the hives were mites of the genera *Bdella*, *Cheyletis* and *Tyroglyphus*, the latter genus being by far the most frequent. These mites were kindly identified for me by Mr C. Warburton.

The larvae of *Lestodiplosis alvei* are comparatively sluggish, and will allow the mites to crawl all over them. At first it was difficult to understand on what they fed, until a passing mite was observed impaled upon the hook-like man-

dibles, which must be capable of a very sudden protrusion. The prey was observed to remain impaled for sometimes an hour or more, after which it was dropped, the liquid contents of the mite having been either sucked out or its tissues liquefied by the larva's saliva. When crowded together the larvae were found to feed on one another.

Growth appeared to be extremely slow, even when isolated larvae were kept with an abundance of mites, and feeding was very rarely observed. The larvae were not particularly common, but seemed to be more plentiful around and within the burrows made by the larvae of *Meliophora grisella* than among the surrounding wax, possibly on account of there being more mites around this larva's faeces than elsewhere. It is also possible that the carnivorous mites *Bdella* and *Cheyletis* might, to some extent, control the increase of *Lestodiplosis alvei* by devouring the first instar larvae.

In order to breed the imagines, portions of the bees' nests were broken up and placed in bell-jars, the wax being kept slightly damp, and, to increase the supply of mites of the genus *Tyroglyphus*, fragments of cheese were added, but it is impossible to state if this made any appreciable difference. Fertile eggs were obtained by placing adult flies in a small bell-jar with a limited supply of wax, which was carefully examined after about four days. The egg is oval, circular in transverse section, pink in colour and tapers slightly posteriorly. It measures approximately 0·2 mm. long and 0·075 mm. in diameter, and the egg shell shows no sculpturing. The first instar larvae, which usually hatch after four or five days, were not able to be kept alive for more than a short time. For the microscopical examination of the larval head capsule, the larvae, after treatment with dilute potash, were mounted in balsam in various positions and examined under a binocular microscope with stereoscopic eyepieces. Staining the chitin with acid fuchsin appeared only to mask the detail in the majority of cases, while attempts to cut transverse and longitudinal serial sections of the minute head proved a failure. No information was obtained as to whether *Lestodiplosis alvei* is entirely restricted to beehives or whether its presence there was accidental—the eggs, or young larvae, having been brought in by the bees, or some other inhabitant of the hive—for the mites which form its food almost certainly occur in other habitats.

B. The larva

I. *General description*

The larva of *Lestodiplosis alvei* (Fig. 1) is similar in shape to larvae of other species of this genus (cf. Kieffer, 1900). The colour varies from rose pink to orange, and the conspicuous fat body is white. The adult larva averages 2·5× 0·6 mm., and the newly hatched larva is 0·45 mm. long and 0·1 mm. wide. As in other Cecidomyid larvae the body is composed of the head, the supernumerary or extra segment, the pro-, meso- and metathoracic segments, and nine abdominal segments. Only newly hatched and adult larvae were

examined, and no information was obtained as to whether *L. alvei* possesses the usual number of four moults. In the newly hatched larva the respiratory system is metapneustic, having only one pair of spiracles situated dorsally on the eighth abdominal segment. In the adult larva the respiratory system is peripneustic with one pair of spiracles on every segment except the supernumerary segment, meso- and metathorax, and last abdominal or anal segment. The spiracles are placed laterally on each side of the segments, the eighth abdominal pair (Fig. 6, *Sp.*) being situated more dorsal than the others. The spiracles (Figs. 4, 6, *Sp.*) are similar in structure to those described by Metcalf (1933) in *Dasyneura leguminicola*.

The fat body (Figs. 2, 3, *F.B.*), which can be seen by transparency, occupies the same positions as described in the larva of *Cecidomyia destructor* (Marchal, 1897) and *Dasyneura leguminicola* (Metcalf, 1933). The lateral lobes, however, are usually confluent, and in some specimens the lateral and ventral lobes run into one another at intervals (Fig. 2). The cuticle is provided with setae, pseudopods and very minute warts, and is frequently traversed by longitudinal and transversely running furrows. The supernumerary segment bears on its dorsal surface a single eyespot, and both this segment and the head can be retracted into the prothorax.

As usual in larvae of this genus there is no sternal spatula. The anus is triangular and situated just anterior to the ventral swellings on the ninth abdominal segment (Fig. 5, *As.*). It is bordered anteriorly by three chitinous flaps, two lateral and one median (Fig. 5, *Fl.*).

II. *The pseudopods*

The pseudopods (Fig. 4, *P.*) are lightly chitinised fleshy protuberances approximately 0·1 mm. long, which arise from ventral swellings on those segments which bear them. They decrease in diameter from their base downwards and terminate in conical-shaped swellings which are fringed with extremely fine hairs. The distribution of the pseudopods is as follows: they are absent on the supernumerary segment, the prothoracic, and the eighth and ninth abdominal segments. Two of them are present on the meso- and metathorax, and three on each of the first seven abdominal segments (Figs. 1, 4, *P.*). Kieffer (1900) mentions the above arrangement and structure of the pseudopods in larvae of this genus, and considers them to represent modified papillae. He figures a pseudopod of a species of *Lestodiplosis* and the ventral surface of the metathorax and first abdominal segments, which in error he labels as the first two thoracic segments. He states that similar pseudopods occur in the larvae of the genera *Arthrocnodax* and *Rubsaamenia*. On the ninth abdominal segment there are two ill-defined blunt swellings just posterior to the anus (Figs. 5, 6, *Sw.*).

III. *The arrangement of the setae and warts* (see Figs. 1, 4, 5 and 6)

Kieffer (1900) mentions several kinds of papillae which occur in Cecidomyid larvae, but the only kind occurring in *Lestodiplosis alvei* are his papillae with

setae. In *L. alvei* the supernumerary segment bears no setae. Dorsally the prothoracic segment bears three setae in a transverse row on either side of the mid-dorsal line; the middle pair is, however, slightly shorter than the others and set slightly more posterior. Laterally, on each side, there are two setae of

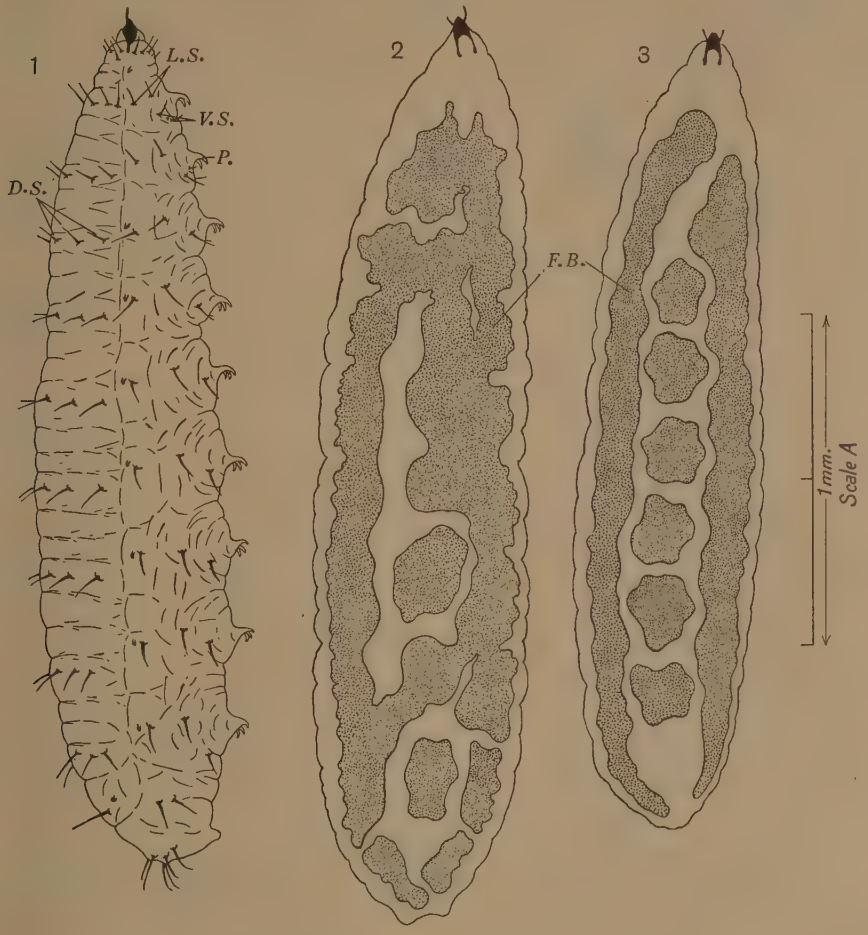

Fig. 1. *Lestodiplosis alvei* Barnes. Lateral view of an adult larva. Figs. 2, 3. Ventral views of adult larvae showing the positions of the fat body.

equal length, one above the other, and situated slightly anterior to the prothoracic spiracle (Figs. 1, 4, *L.S.*). A similar dorsal and lateral arrangement of the setae exists on all the other segments, except dorsally on the eighth and ninth, and laterally on the ninth abdominal segments. Ventrally there are three setae on each side of the mid-ventral line (Fig. 4, *V.S.*). The most lateral

pair are approximately the same length as the other setae, the middle pair are extremely small and set slightly posterior, while the inner pair are slightly longer than the middle pair and are set more posterior still. On the meso- and metathoracic segments the three pairs of ventral setae are arranged posteriorly around the base of the ventral protuberance from which the pseudopods arise (Fig. 4). On the abdominal segments (1–7), the middle and inner

Fig. 4. *Lestodiplosis alvei* Barnes. Ventral view of the head and first five segments of an adult larva. Fig. 5. Ventral view of last abdominal segment (adult larva). Fig. 6. Lateral view of the last two abdominal segments (adult larva). Fig. 7. Pseudopods on the first abdominal segment of a first instar larva.

pairs of ventral setae are absent, while the outer pair remain in the same position as on the thoracic segments. Laterally the more dorsal seta is situated just below the spiracle, while the dorsal setae are all in a straight line transversely across the centre of the segments. The eighth abdominal segment has only two dorsal setae (Fig. 6, *D.S.*), which are situated slightly posterior to the spiracle, and no ventral setae. Around the posterior extremity is a transverse row of four long and curved setae, and a little dorsal to these a pair

of long setae situated between the outer and inner setae of the row below (Figs. 5, 6, *P.S.*). All these six posterior setae are set on pronounced papillae. Barnes (1928) figures a similar arrangement of posterior setae on the larva of *L. pisi*.

The only other dermal structures, besides the setae, occurring on the cuticle of *L. alvei*, are certain minute spiny warts (Fig. 4, *W.*). Kieffer (1900) mentions four kinds of warts as occurring on Cecidomyid larvae, but the only kind found on *L. alvei* are apparently his warts with spines. These appear as minute serrations on the cuticle in irregular broken transverse rows around the bases of the ventral protuberances which bear the pseudopods, and occasionally on the pseudopods themselves.

Around the ventral swellings on the last abdominal segment there are numerous transverse rows of these warts (Fig. 5, *W.*), while around the anterior border of the anus, at the bases of the chitinous flaps, is a single row of large spines which might almost be termed minute setae (Fig. 5, *A.S.*).

IV. *The head capsule* (see Figs. 8–11)

The head capsule consists of an elongated, and ill-defined, chitinous cone of varying thickness. In the adult larva this measures approximately 0·13 mm. in total length and 0·055 mm. in height and width. Laterally the base of this cone is produced posteriorly into two strongly chitinised horns (Figs. 8–11, *H.*). Ventrally two thin fin-like flanges of chitin arise from these lateral horns, about 0·025 mm. from their posterior extremities (Figs. 8–10, *F.*). These fins run forward and downward, eventually meeting in the mid-ventral line, at a point approximately one-third of the total length of the complete head capsule from its anterior end. The ventral and lateral sides of the cone are formed by the fusion of these fins, this being in the form of a thin plate posteriorly, but with local thickenings anteriorly to form presumably the labium (Figs. 9, 10, *Lm.*). On the inner side of these lateral fins can be seen dorso-ventral running ridges, or local thickenings, possibly for muscular attachment, which terminate ventrally at the upper edge of an oval area of thinner chitin, which extends anteriorly almost to the point of juncture of the two fins (Figs. 9, 11, *R.* and *Ov.*). Dorsally two strongly chitinised triangular projections arise from each lateral horn (Figs. 8, 9, *T.*). These join at their apices in the mid-dorsal line at about the centre of the head capsule. In the centre of each of these triangular projections is a minute foramen (Figs. 8, 9, *Fo.*). Anterior to these projections is a narrow area of thin chitin (Figs. 8, 9, *T.C.*), which extends forward along the mid-dorsal line as a narrow plate to join a thickly chitinised region (Figs. 8, 9, *L.B.P.*), which bears on its anterior side the triangular labrum (Figs. 8, 9, *L.* and Fig. 15). On either side of this thin median plate are two large oval openings (Figs. 8–10, *Oo.*), which are bordered posteriorly and laterally by extensions of the thinly chitinised area mentioned above, and anteriorly by the thick labrum-bearing plate (Figs. 8, 9, *L.B.P.*). The oesophagus is a thickly chitinised tube, which can easily be seen to pass from the head cone nearly to the ends of

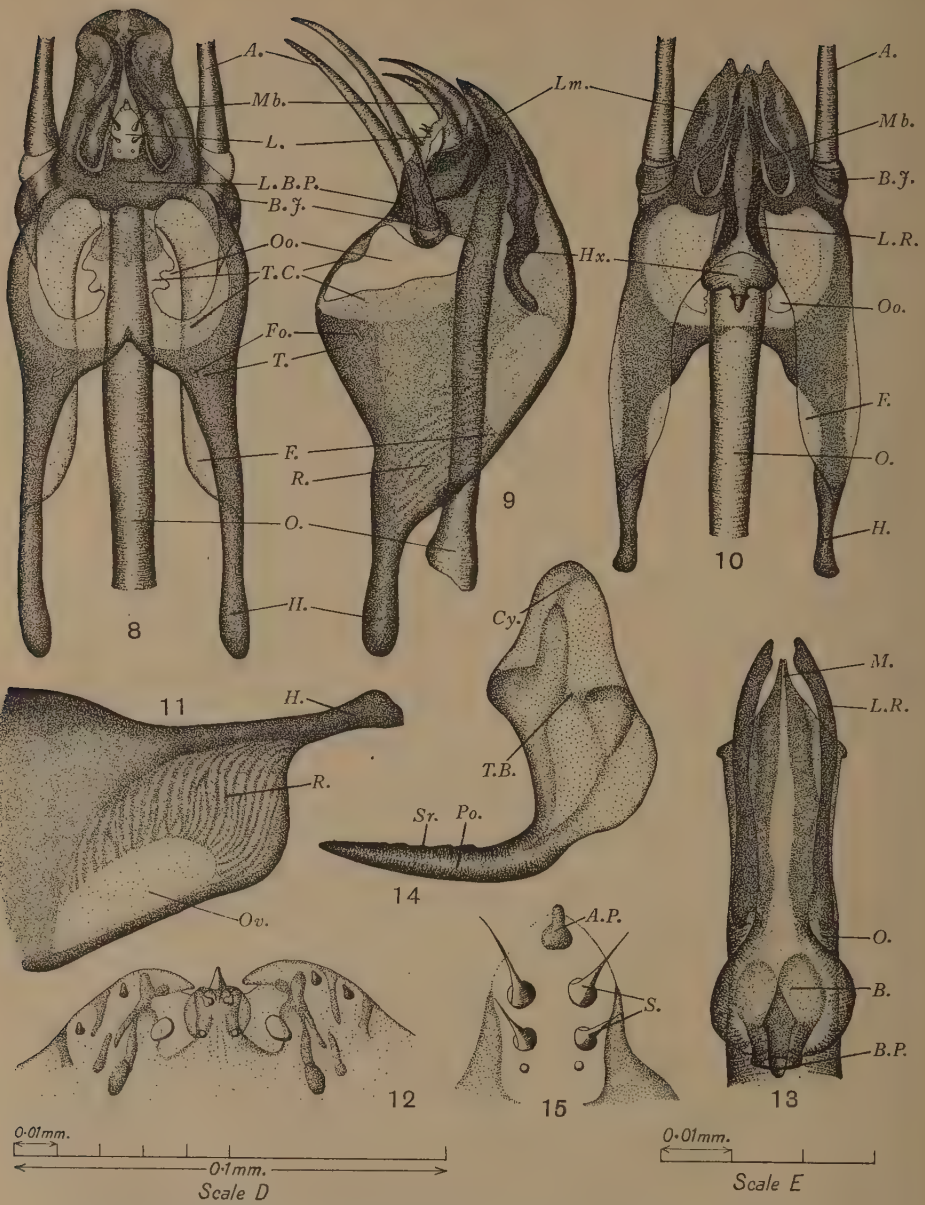

Fig. 8. *Lestodiplosis alvei* Barnes. Head capsule of an adult larva. Dorsal view. Fig. 9. Head capsule, lateral view. Fig. 10. Head capsule, ventral view. (In Figs. 8, 10 the terminal joint of the antennae is cut off short.) Fig. 11. Inner side of the posterior region of a head capsule (somewhat flattened out). Fig. 12. Labium and surrounding chitin. Fig. 13. Hypopharynx, ventral view. Fig. 14. Mandible, lateral view. Fig. 15. Labrum, dorsal view.

the backwardly projecting horns, where it joins the fore intestine (Figs. 8–10, *O.*). In lateral and ventral views a peculiar structure, probably the hypopharynx (Figs. 9, 10, *Hx.*), can be seen lying ventral to the oesophagus. The antennae are borne on lateral outgrowths from the head capsule (Figs. 8–10, *A.*), at the posterior border of the thick labrum-bearing plate.

V. *The mouth-parts and head structures* (see Figs. 12–15)

Ventrally the floor of the head cone, as stated previously, consists of the prolongation of the ventral fins. Very little detail can be seen in this plate, except localised thickenings, and a few that are presumably sense pits or papillae. Anteriorly, however, a minute anterior projecting median papilla was observed, which appeared to be borne on a thickened rectangular base. The whole of this ventral plate possibly represents the labium (Figs. 9, 10, *Lm.*, and 12).

Dorsal to this, and lying ventral to the oesophagus, is a peculiar structure which is probably the hypopharynx (Figs. 9, 10, *Hx.*, and 13). It consists posteriorly of a dorso-ventrally flattened thinly chitinised bulb, which is possibly a salivary reservoir or pump (Fig. 13, *B.*). Projecting posteriorly from this bulb is a short blunt projection, whose base appears to be surrounded by the bulb (Fig. 13, *B.P.*), which is curved ventrally. Anteriorly a short, wide tube leads from this bulb and runs into the ventral wall of the oesophagus just posterior to the mouth.

The minute mouth opening is situated on the apex of a thickly chitinised cone (Fig. 13, *M.*). Arising at a point approximately at the base of the hypopharynx bulb, and running forward and slightly ventral on either side of the oesophagus, are two chitinous rods (Figs. 10, 13, *L.R.*). These rods extend forward and bend inward slightly in front of the mouth. From their position they may represent the maxillae, or may form part of the hypopharynx.

Dorsal to the oesophagus lie paired laterally placed hook-like structures, which are probably the mandibles (Figs. 8–10, *Mb.*, and 14). They consist of a thickly chitinised pointed portion (Fig. 14, *Po.*), slightly convex anteriorly, and with minute serrations on their inner concave surfaces (Fig. 14, *Sr.*). They have large triangular bases (Fig. 14, *T.B.*), which lie roughly at right angles to the anterior portions, the apices of these triangular bases forming condyles (Fig. 14, *Cy.*), which appear to articulate with the under-surface of the thick labrum-bearing plate. The mandibles lie slightly obliquely in the head capsule with their condyles wide apart, and with the bases of their curved portions almost meeting in the mid-dorsal line. The points can be protruded dorsally and laterally outside the head capsule. It is with these structures that the larvae pierce and hold the mites upon which they feed. The mandibles are by far the largest and most conspicuous of the head appendages.

The labrum (Figs. 8, 9, *L.*, and 15) is a thin roughly triangular plate, with its apex anterior, lying dorsal to the supposed mandibles and between their bases. It bears on its dorsal surface a single minute papilla (Fig. 15, *A.P.*), followed by two pairs of extremely small setae (Fig. 15, *S.*), and a pair of sense pits. The

labrum joins the thick labrum-bearing plate at its base, which also sends thickened projections half-way up its two sides.

The antennae (Figs. 8–10, *A.*) are two-jointed and approximately 0·07 mm. long, the terminal joint being by far the longest. The basal joint (Figs. 8–10, *B.J.*) is very short and closely connected to the head capsule.

VI. *The homology of the head capsule and mouth-parts of the larva of* Lestodiplosis alvei *with other Cecidomyid larvae*

Most of the authors on Cecidomyid larvae have either neglected describing the head capsule and mouth-parts entirely, or confined their description to a very brief account. The extremely minute size of the capsule being the reason of their failure to differentiate the detail of its structure. In fact the head capsules of numerous species were often figured as being composed of a series of chitinous rings and rods.

Ratzeburg (1841) was the first to examine the larval head capsule in the case of *Cecidomyia pini* and *C. brachyntera*, but was unable to find the mouth or to explain the structure of the head capsule. Busgen (1895) also could not find mouth-parts in the larva of *Mikiola* (*Cecidomyia*) *fagi*, but considers that these larvae pierce plant tissues by means of a very fine stylet.

In *Cecidomyia destructor*, Marchal (1897) finds a dorsal arch, which he describes as a kind of labrum, under this two pairs of chitinous nodules, then two blunt pieces on either side of the mouth, whose posterior ends are produced internally to form a chitinous box, which is bent downwards and opens into the oesophagus in front. Finally comes a median stylet (ligula), which carries the salivary duct, and which is placed ventral and median to the box.

Rubsaamen (1899) mentions that the head capsules of carnivorous Cecidomyid larvae have longer backwardly projecting horns than the phytophagous species, and figures heads of *Arthrocnodax* and *Arnoldia* as examples.

Kieffer (1900) figures the head capsules of *Mycodiplosis reaumuri* and *Atriosema aceris*. The head capsules of both these species consist of two backwardly projecting arms, which unite ventrally to form a horseshoe; from the ends of these arms a loop is given off, which extends dorsally and gives off two dorsal thickenings. Anterior to the horseshoe the structure is difficult to follow from his drawings and description, except that dorsally there is a longitudinal and median piece, under this there is on each side a longitudinal and lateral piece, then the oesophagus tube, and ventrally two parallel pieces, which arise from the arch of the horseshoe, and which bear at their base a minute ligula. He states that the degree of chitinisation of the head capsule varies greatly with the species. He could not find the mouth opening.

Williams (1910) states that the head of *Cecidomyia resinicoloides* consists of a broad irregular ring from which a pair of prong-like rods project well into the supernumerary segment. No details of mouth-parts are given.

Chaine (1913) mentions the conical nature of the head capsule in *Monarthropalpus buxi*, but gives no figures or detailed description. Hamilton

(1925) briefly mentions two pairs of lateral appendages, mandibles and maxillae, in *M. buxi*.

Hasemann (1930) very briefly describes the head of *Phytophaga* (*Cecidomyia*) *destructor* as consisting of two pairs of brown chitinous structures, and a rather distinct forked structure, which is probably the labium. He mentions the backwardly projecting horns, but it is not clear if he considers them as part of the labium. It is difficult to homologise the structures in his account of the head capsule with those of Marchal's (1897) description of the same species.

Metcalf (1933) states that the head of *Dasyneura leguminicola* is supported by a ring of chitin, which has two lateral prongs directed posteriorly. The mouth is ventral and bordered by a dorsal appendage, a ventral appendage and a pair of lateral appendages. In order to conform with Kellogg's (1899) hypothesis that the mouth-parts of the imago develop in close relation to those of the larva, these appendages are therefore termed respectively labrum-epipharynx, labium and maxillary palps, there being no mandibles in the imago.

Among the Nematocera, the Cecidomyidae alone are considered to share the hemicephalous condition of the larval head with the Tipulidae. In the genus *Lestodiplosis*, and probably in several others, this hemicephalous condition is conspicuous on account of the great development of the posterior part of the head capsule and lateral horns, which giving a greater surface for muscular attachments enables the larva to hold its living prey. However, on examining some larvae of a species of *Joannisia*, obtained from rotten wood, a false encephalous condition of the head was at first observed, owing to the extreme smallness of the lateral horns with none of the developments characteristic of the posterior region of the head capsule in *Lestodiplosis alvei*. It is, however, clear, as mentioned by Kieffer (1900), that the head capsules of Cecidomyid larvae are modelled to some extent on a constant plan. Even if considering the genus *Lestodiplosis* to be specialised carnivorous feeders, certain resemblances with species of other genera are apparent.

The lateral backwardly projecting horns in *Lestodiplosis alvei* evidently correspond to the horns of Kieffer's (1900, *Mycodiplosis reaumuri*) horseshoe, the two lateral prongs of Williams (1910, *Cecidomyia resinicoloides*), Metcalf (1933, *Dasyneura leguminicola*), Rubsaamen (1899) and Hasemann (1930, *Phytophaga destructor*), and perhaps to the two internally projecting pieces of Marchal (1897, *Cecidomyia destructor*). The junction of the ventral fins corresponds probably to the arch of Kieffer's horseshoe and part of the chitinous ring of Metcalf, while Kieffer's dorsal projections in the head of *M. reaumuri* may correspond to the dorsal triangular projections.

As regards the mouth-parts very little information is forthcoming. The labrum of *Lestodiplosis alvei* may correspond to Marchal's labrum, Kieffer's longitudinal and median piece, and Metcalf's labrum-epipharynx. The mandible-like structures possibly correspond to Marchal's blunt pieces on either side of the mouth, and to Kieffer's longitudinal and lateral pieces. The supposed hypopharynx, however, appears to be the mouth-part that is most easily

traced, for this almost certainly corresponds to Marchal's median stylet (ligula), while Kieffer's two ventral parallel pieces may possibly represent the lateral rods.

On account of the entire lack of knowledge regarding the larval mouth-parts of other representatives of this family, it is impossible to draw homologies of these structures with those of other Nematocerous larvae. The terminology of the regions of the head capsule and mouth-parts of the few Cecidomyid larvae which have been described are therefore peculiar to each author, depending on what he considers the various regions and parts to represent, even the antennae are termed antennules by Marchal, palps by Kieffer and tentacles by Metcalf. The fact that the mandibles of larval Nematocera are horizontally opposed, does not entirely rule out the supposition that the hook-like structures in *L. alvei* are not true mandibles, for the position of their bases and condyles in the head capsule, and the angle and curvature of the pointed portions in relation to their bases, shows that the dorso-lateral protrusion of the points follows from a horizontal movement of the bases, or rather that the horizontal movement is translated into a dorso-lateral protrusion of the points on account of their touching on the mid-dorsal line.

VII. *The first instar larva*

The first instar larva differs only very slightly from the adult. The head capsule, which is only 0·055 mm. long and 0·035 mm. wide, appears exactly similar to that of the adult, although not so heavily chitinised; no setae, however, could be identified on the labrum. The arrangement of the setae and pseudopods is also similar, but the structure of the latter is somewhat different, resembling in this instar long and blunt-ended setae (Fig. 7, *P.*). No rows of warts could be distinguished upon the ventral protuberances. The first instar larva is metapneustic with the only pair of spiracles on the eighth abdominal segment. The spiracles are large and conspicuous and occupy the same position as in the adult larva.

C. The pupa

The pupa, which measures approximately 1·8 mm. long and 0·6 mm. wide, shows no peculiar features, and is very similar to the pupae of other Cecidomyidae as figured by Marchal (1897), Kieffer (1900) and others. Anteriorly there are two lateral triangular projections on the head, each of which bears a long seta, and posteriorly to these are two long setae.

Pupation occurs usually between two layers of wax and there appears to be no cocoon. Very few pupae were found.

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Scales of the Figures

Figs. 1–3	Scale A.
Figs. 4–6	Scale B.
Fig. 7	Scale C.
Figs. 8–11	Scale D.
Figs. 12–15	Scale E.

Lettering common to all the Figures

A. antennae; *A.P.* apical papilla of the labrum; *A.Sg.* abdominal segment; *A.S.* anal setae; *As.* anus; *B.* bulb of the hypopharynx; *B.J.* basal joint of the antennae; *B.P.* the blunt posterior projection on the hypopharynx bulb; *Cy.* condyle of the mandible; *D.S.* dorsal setae; *F.* ventral fins of the head capsule; *F.B.* fat body; *Fl.* chitinous flaps which border the anus posteriorly; *Fo.* the minute foramina in the head capsule; *H.* posterior horns of the head capsule; *Hd.* head; *Hx.* hypopharynx; *L.* labrum; *L.B.P.* labrum-bearing plate; *Lm.* labium; *L.R.* lateral rods of the hypopharynx (maxillae); *L.S.* lateral setae; *M.* mouth opening; *Mb.* mandible; *M.T.* mesothorax; *Mt.T.* metathorax; *O.* oesophagus; *Oo.* large oval openings in the head capsule; *Ov.* oval area of clear chitin on ventral fins of head capsule; *P.* pseudopods; *Po.* pointed portion of mandibles; *P.S.* posterior setae; *P.T.* prothorax; *R.* ridges on ventral fins of head capsule; *S.* setae; *Sp.* spiracle; *Sr.* serrations on points of mandibles; *Su.* supernumerary segment; *Sw.* swellings on ninth abdominal segment; *T.* dorsal triangular projections on the head capsule; *T.B.* triangular bases of the mandibles; *T.C.* area of thin chitin on dorsal region of head capsule; *V.S.* ventral setae; *W.* warts.

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LESTODIPLOSIS ALVEI SP.N. (DIPTERA, CECIDOMYIDAE)

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Lestodiplosis alvei sp.n.

Male. Length about 1·5 mm. Antennae slightly longer than head, thorax and abdomen: 2+12, basal enlargement of flagellar segments with regular ring of moderately long stout setae and one ring of circumfila, distal enlargement with two rings of circumfila and irregular ring of stout setae, loops of circumfila regular and moderately long, those on basal enlargement extending about one-third length of stem, distal ring on distal enlargement extending about two-thirds length of neck; 1st and 2nd flagellar segments fused; 3rd flagellar segment with stem about three times as long as broad, neck slightly longer, about three-and-a-half times as long as broad, distal end slightly darkened as on all flagellar segments; 10th flagellar segment with stem about four times as long as broad, neck slightly longer in proportion. Palpi quadriarticulate with few setae: proximal segment quadrate, 2nd just over three times as long as broad, 3rd about three times as long as broad, the same width as 2nd, distal segment about four times as long as broad, slightly narrower than two previous segments. Thorax brown. Wings hyaline; 3rd vein reaching margin at apex of wing. Legs hairy; claws moderately curved, simple; empodium slightly shorter than claws. Genitalia: basal clasp segment long narrow, small basal lobe prominent; distal clasp segment long narrow, darkened; dorsal lamella moderate length and breadth, distinctly longer than ventral lamella, deeply emarginate, each lobe rounded; ventral lamella comparatively short but broad; style long, moderately stout.

Cotypes: Cecid. 2026–30 and 2335–6 inclusive.

Female. Length about 2 mm. Antennae slightly shorter than thorax and abdomen: circumfila applied, basal ring of setae regular, setae on 1st and 2nd flagellar segments long, extending to base of next segment, on other segments much shorter, distal ring of setae irregular; neck of 3rd flagellar segment nearly four-and-a-half times as long as broad, neck of 10th flagellar segment about four times as long as broad, necks of proximal segments slightly darkened. Palpi: proximal segment quadrate, 2nd just over three times as long as broad, slightly swollen, 3rd about four times as long as broad, distal segment about five

times as long as broad. Wings hyaline. Ovipositor lamelliform, lamellae about three-quarters as broad as long. Otherwise about as in male.

Cotypes: Cecid. 2031, 2337 and 2338.

Habitat: reared from larvae found in old beehive infected with mites, Selehurst, Horsham, Sussex (G. W. Otter).

Note. This is the second gall midge to be found in beehives. The previous species, *Arthrocnodax apiphila* Felt, was reared from larvae feeding in mite-infested material and excrement of old bee combs received from California. The male of *A. apiphila* was described in 1908 (*N.Y. St. Mus. Bull.* No. 124, p. 301) and both sexes were described in 1921 (*loc. cit.* No. 231–2, pp. 81, 87–89, figs. 4–6). While the larvae of most species of *Arthrocnodax* are mite-eaters, the larvae of most species of *Lestodiplosis* are predaceous on other gall midge larvae, although three species are known to feed probably on mites. Information regarding gall midges as enemies of mites has recently been collected together (*Bull. Ent. Res.* **24**, 1933, 215–28).

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